Survival of rhizobia in Saskatchewan soils as influenced by freeze/thaw cycling.

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Abstract

Rhizobium leguminosarum biovar *viceae* are not native to Saskatchewan, but numbers of rhizobia in excess of 10^4 g^{-1} soil have been observed in fields previously grown to inoculated field pea (*Pisum sativum* L.). Modified immunoblot and ELISA techniques utilizing strain-specific polyclonal antibodies were used to monitor the survival of rhizobial populations under freeze/thaw conditions in field and controlled environment studies. Total rhizobial populations tended to decrease with each freeze/thaw cycle, but there were not always significant differences between pre- and post-freeze rhizobial populations. Total rhizobial numbers tended to be affected more by a fallow period then by the freeze/thaw cycle itself. The effect of soil moisture content at freezing on total populations varied with strain. The proportion of the total rhizobial population able to nodulate field pea was not affected by the freeze/thaw cycle, but a decrease during the fallow period was observed.

Introduction

Field pea (*Pisum sativum*) forms a symbiosis with the rhizobium *Rhizobium leguminosarum* biovar *viceae*. Neither the crop nor the bacteria are native to the Canadian prairies. The cold Saskatchewan environment is not considered favorable for bacterial survival (Bailey 1989; Campbell et al. 1970). Depending on snow cover the winter soil temperature may drop below -10°C at the 5cm depth. A study was initiated in 1990 to identify factors which affect the survival of rhizobia under Saskatchewan environmental conditions. The main objective of this study was to determine how rhizobial populations would be affected by freeze/thaw cycling.

Materials and Methods

The study was comprised of field and freezer components. The field sites were used to monitor the population change of *R. leguminosarum* over three growing seasons (two winters). The freezer studies were used to monitor the change in population of *R. leguminosarum* following freeze/thaw cycles under different soil moistures and textures.

Rhizobia Enumeration

Polyclonal antibodies were created against vegetative *R. leguminosarum* cells and cross-adsorbed with heterologous cells to produce antisera specific to either isolate C-1 (isolated in Manitoba from fababean (*Vicia faba*)), TA101 (isolated from Australian soil) or USDA 2470 (recommended by the USDA for field pea). The total rhizobial population of either C-1, TA101, or USDA 2470 was determined from serial dilutions of bulk or rhizosphere soil extracts plated on selective media (against fungi, actinomycetes, Gram positive bacteria and some Gram negative bacteria). Plates were grown at 28°C for 4 days and assayed by a modified Western Blot termed an Immuno-Blot. The proportion of the total population which would nodulate the host crop, termed the effective population, was determined using a modified most-probable-number (MPN) technique termed an Immuno-MPN. This assay utilized serial-diluted soil extracts which were inoculated onto surface-sterilized field pea seed, germinated and grown in a sterile environment for 21 days. Nodules from infected roots were collected and typed using an Enzyme Immuno-sorbent Assay (ELISA).

Field Component

Between 1990 and 1992 sites were selected at Biggar (Dark Brown Chernozem) and Prince Albert (Black Chernozem) to monitor seasonal rhizobial population changes. The Biggar site had produced an inoculated field pea crop in 1986, and the Prince Albert site had produced a field pea crop in 1988. Both were first time pulse crops. Field pea, inoculated with greater than 10^5 viable rhizobia seed^{-1}, was grown at both sites in 1990 and 1991. Wheat was grown in 1992. The rhizobial population was determined from bulk soil samples prior to planting in 1990 and 1991 and from bulk soil samples in the spring and fall of 1992. The rhizobial population was determined from rhizosphere soil samples in the fall of 1990 and 1991.

Freezer Component - Experiment I

R. leguminosarum biovar *viceae* isolate TA101 was added to a loamy sand soil (Dark Brown Chernozem free of indigenous rhizobia) in combination with isolate C-1 as a multi-strain inoculant. Three moisture regimes were imposed on the soil; dry (-1.5 MPa), moist (-0.25 MPa) and wet (-0.03 MPa). The soil was then incubated at 20°C for two weeks prior to initiation of the first freeze/thaw cycle. Soil temperature was then reduced at the rate of 1/100°C min^{-1} to -10°C and was maintained at that temperature for 10 days. The soil temperature was then raised at 1/100°C min^{-1} to +20°C. A sample was taken and the soil refrozen to -10°C, maintained for 40 days then raised to +20°C and sampled. The soil was then kept at +20°C for 35 days (representing a fallow period) and re-sampled. Bulk soil samples were enumerated for total and effective rhizobial populations.

Freezer Component - Freezer Experiment II

Isolate USDA 2470 was added to a loamy sand soil (Dark Brown Chernozem free of indigenous rhizobia) in combination with isolate C-1 as a multi-strain inoculant. Two soils of differing textures (loamy sand - 56% sand, 30% silt, 14% clay ; clay - 8% sand, 33% silt, 59% clay) plus a saline soil (22 mS/cm using a 1:1 soil:water conductivity test) were used. The soils were incubated for two weeks prior to initiation of the first freeze/thaw cycle. Soil temperature was reduced at the rate of 1/100°C min^{-1} to -22°C and maintained for 4 days. Soil temperature was then increased at 1/100°C min^{-1} to +10°C. The soils were sampled and then refrozen to -22°C, maintained for 10 days, then raised to +10°C and re-sampled. Bulk soil samples were enumerated for total and effective rhizobial populations.

Note 1: Only results obtained from isolate C-1 will be discussed.

Note 2: Results from the saline soil will not be discussed as rhizobial die-off occurred within the 14 day incubation period.

Results and Discussion

Field Component

The sites at Biggar and Prince Albert were monitored from 1990 until 1992. Each site had a high initial population (Table 1). The effective population increased during the growing season for both 1990 and 1991 in the presence of the host crop. The winter of 1990/91 was harsh at the Biggar location. There was sparse snow cover and temperatures at the 5 cm soil depth dropped to below -10°C. There was a continuous deep snow cover at the Prince Albert location and soil temperature at the 5 cm depth did not go below -2°C. The effective population at the Biggar location declined, while at the Prince Albert location it remained at the same level as the previous year. Only the Biggar location was monitored in 1992. The 1991/92 winter was mild, with a deep snow cover and a low soil temperature of -1°C at the 5 cm depth. Similar to the Prince Albert location the previous year population levels were maintained from Fall to Spring. This indicated that the degree of harshness over winter may play a role in rhizobial survival.

Table 1. Effective population of rhizobia at the Biggar and Prince Albert locations between 1990 and 1992.

	Biggar	Prince Albert
	Log rhizobia g^{-1} soil	
Spring 1990 (pre-plant)	4.4$^{\beta}$	4.6
Fall 1990 (Flowering)	5.1	3.9
Spring 1991 (pre-plant)	4.4	4.7
Fall 1991 (Flowering)	6.1	5.7
Spring 1992	6.1	nd$^{\kappa}$
Fall 1992	5.0	nd

β - 95% fiducial limits - ± 0.42

κ - not determined.

Freezer Component - Experiment I:

The total population remained stable following the first freeze/thaw cycle, declined following the second freeze/thaw cycle and remained stable throughout the fallow period. The effective population was maintained during the first freeze/thaw cycle, but declined during the second cycle and fallow period. There were not distinct effects of soil moisture content on the survival of rhizobia. Wet conditions appeared to favor total numbers, while moist conditions appeared to favor the effective population. It appeared that moist, rather than dry conditions were more favorable to rhizobial survival.

The proportion of effective rhizobia did not change throughout the freeze/thaw cycles, but a significant decrease occurred during the simulated fallow period (Table 3). Because the total rhizobial population did not change in the fallow period compared to the final freeze/thaw cycle it may indicate that effective rhizobia are less competitive in a fallow soil.

Table 2. Change in the total and effective rhizobial population as influenced by freeze/thaw cycling and soil moisture.

Sampling Period	Total Population		Effective Population	
	Log rhizobia g^{-1} soil			
Initial	6.2	a$^{\beta}$	5.0	b$^{\kappa}$
Cycle 1	5.9	a	5.5	a
Cycle 2	4.8	b	4.0	c
Fallow	5.1	b	3.1	d
Moisture				
Dry	6.0	a	4.6	b
Moist	5.4	b	5.4	a
Wet	5.2	b	4.1	c

B - treatments followed by the same letter are not significantly different at the 5% level using an F-protected LSD.

κ - 95% fiducial level of ±0.42 log units.

Table 3. Moisture effect on the ratio of total to effective rhizobial population following two freeze/thaw cycles and a fallow period.

	Initial	---Cycle--- 1st	2nd	Fallow
Time	70	60	60	2200
Dry	10	80	30	3200
Moist	180	50	10	3200
Wet	20	40	140	600

Freezer Component - Experiment II:

There was no difference between the loamy sand and clay soil for rhizobial survival (Table 4). The total rhizobial population remained stable following one freeze/thaw cycle and decreased following the second cycle. The effective population remained stable (Table 5). The proportion of effective rhizobia did not change following one freeze/thaw cycle. This was similar to the first experiment.

Table 4. Change in the total and effective rhizobial population as influenced by freeze/thaw cycling and soil texture.

Sampling Period	Total Population		Effective Population	
	Log rhizobia g^{-1} soil			
Loamy sand				
Initial	6.0	a$^{\beta}$	4.5	b$^{\kappa}$
Cycle 1	5.4	a	4.8	a
Cycle 2	5.0	b	nd$^{\delta}$	
Clay				
Initial	5.5	a$^{\beta}$	4.6	b$^{\kappa}$
Cycle 1	4.7	a	4.4	a
Cycle 2	4.9	b	nd	

B - treatments followed by the same letter are not significantly different at the 5% level using an F-protected LSD.

κ - 95% fiducial level of ±0.42 log units.

δ - not determined.

Table 5. Texture effect on the ratio of total to effective rhizobial population following one freeze/thaw cycle.

	Initial	Cycle 1
Loam	31	4
Clay	9	2

Conclusions

1. The total rhizobial population was reduced by an initial freeze/thaw action but the population stabilized and a further reduction was due to a fallow period.

2. The effective rhizobial population remained stable throughout the freeze/thaw cycles, but declined rapidly during the fallow period.

3. There were no definitive trends in the survival of rhizobia at the different soil moisture contents, although it appeared that moist conditions were more favorable.

4. Soil texture did not affect rhizobial survival.

References

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