The influence of host and non-host crops on the rhizobial population of the root rhizosphere. D. FEINDEL[1]*, C. VAN KESSEL[1] and L. NELSON[2], [1]Univ. of Saskatchewan, [2]Plant Biotechnology Institute (NRC), Saskatoon, Saskatchewan.

Abstract

Field pea (*Pisum sativum*), a new crop to the Dark Brown and Black Chernozemic soil zones of Saskatchewan, forms a symbiosis with *Rhizobium leguminosarum* biovar *viceae*. This rhizobium is not native to the region, yet numbers in excess of 10^4 g^{-1} soil have been observed several years after a single inoculated field pea crop was grown. Modified immunoblot and ELISA techniques utilizing strain-specific polyclonal antibodies were used to monitor the environmental effect of host and nonhost crop on rhizobial populations. The proportion of soil rhizobia able to nodulate pea differed between a competitive and poorly competitive isolate. Numbers of rhizobia declined over time in non-rhizosphere soil and increased in the presence of host plant. Both isolates maintained or increased soil populations from the initial level in the presence of certain non-host plants. The proportion of rhizobia available to nodulate a pea root increased more for the poorly competitive isolate in the presence of host and non-host root systems but did not reach the level of the highly competitive isolate.

Introduction

Field Pea forms a symbiosis with the rhizobium *Rhizobium leguminosarum* biovar *viceae*. Neither the crop nor the bacteria are native to the Canadian prairies. A study was initiated in 1990 to identify factors which affect the survival of rhizobia under Saskatchewan environmental conditions. Current cropping systems often will include grain legumes in the rotation not more than every third or fourth year. Rhizobial populations often increase in the presence of a host crop, but decline and remain stable at a lower population in the absence of the host crop (Kucey and Hynes 1989). The objective of this study was to determine whether rhizobial populations would be maintained in the presence of host and non-host crops.

Materials and Methods

The study was comprised of field and greenhouse components. The field sites were used to monitor the population change of *R. leguminosarum* in the presence of a host crop (field pea). The greenhouse studies were used to monitor the change in population of *R. leguminosarum* in the presence of host (field pea) and non-host (wheat (*Triticum aestivum*, flax (*Linum usitatissimum*), soybean (*Glycine max*)) crops, or in a fallow soil.

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*)) or TA101 (isolated from Australian soil). The total rhizobial population of either C-1 or TA101 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 serially-diluted soil extracts which were inoculated onto surface sterilized field pea seed. The seed was then germinated and grown for 21 days. Nodules from infected roots were collected and typed using an enzyme immuno-sorbent assay (ELISA).

Field Sites

Eight sites were selected in the Dark Brown and Black Chernozemic soil zones of Saskatchewan to survey indigenous rhizobial populations. The indigenous rhizobial population was determined from soil samples collected from the top 15 cm prior to planting.

Between 1990 and 1992 sites were selected at Biggar (Dark Brown Chernozem) and Prince Albert (Black Chernozem) to monitor rhizobial population changes under the influence of the host field pea crop. Inoculated field pea was grown for the first time in 1986 at the Biggar location and in 1988 at the Prince Albert location. Field pea, inoculated with greater than 10^5 viable rhizobia $seed^{-1}$, was grown at both sites in 1990 and 1991 followed by wheat 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.

Greenhouse Experiment

R. leguminosarum biovar *viceae* isolate TA101 was added to a loamy sand soil (Dark Brown Chernozem free of indigenous rhizobia) either as a single strain inoculant or in combination with isolate C-1 as a multi-strain inoculant. The soil was then incubated for two weeks prior to planting. Four crops (field pea, wheat, flax and soybean) were planted, along with a fallow control. Plant roots were collected when the plants were at the full flower stage (approximately 70 days after planting). Rhizosphere soil (bulk soil from the fallow control) was enumerated for total and effective rhizobial populations.

Results and Discussion

Field Component

A survey of sites (Tables 1 and 2) indicated that there were low to zero rhizobia present capable of nodulating field pea prior to inoculation with field pea. The data also indicated that sites with a history of field pea maintained a high rhizobial population.

The sites at Biggar and Prince Albert were monitored from 1990 until 1992. Each site had a high effective population (Table 2). The effective population tended to increase during the cropping season for both 1990 and 1991 in the presence of the host crop. During 1992 when a non-host wheat crop was grown (for the Biggar location only) the effective population declined, although overall numbers were still very high. This indicated that there is a beneficial host crop effect, but it also indicated that although numbers decreased, there was not a short term detrimental non-host effect.

Table 1. Effective population of rhizobia from field pea sites in Saskatchewan.

Site	Soil Zone	Field Pea Previously Grown	Rhizobia Numbers Log rhizobia g^{-1} soil
1	Dark Brown	No	0.0β
2	Dark Brown	Noγ	0.0
3	Dark Brown	No	1.0
4	Dark Brown	Noκ	3.0
5	Black	Noκ	0.3
6	Black	Yes (10)δ	4.8
7	Black	No	0.6
8	Black	No	0.6

β - Log Rhizobia g^{-1} oven-dry soil.
γ - Uninoculated field pea grown once previously.
κ - Adjacent field grew field pea previously.
()δ - Years since last field pea crop.

Table 2. 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)	4.4β	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	ndκ
Fall 1992	5.0	nd

β - 95% fiducial limits - ± 0.42

κ - not determined

Greenhouse Component

Total populations of the two isolates responded differently to host and non-host crop (Table 3). The total population of C-1 remained stable in the presence of either the host or non-host crop, but decreased in the absence of any crop. Total numbers of TA101 increased in the presence of the host crop, and the non-host legume (soybean). The other non-host non-legume crops, flax and wheat, maintained the population at the initial level, while the population of TA101 in the fallow control declined. Even though the two isolates responded differently, TA101 was more variable. It is evident that for the crops tested, the presence of a crop (root system) was enough to maintain the rhizobial population at the same level as at the time of seeding.

The effective population (Table 4) was even more variable. In the presence of the host crop the effective population was either maintained (C-1) or greatly increased (TA101). The effective population of TA101 was more variable, but the presence of a non-host crop (root system) was enough to maintain or increase the rhizobial population. The

observation that TA101 exhibited the greatest increase in effective populations is misleading. The proportion of rhizobia able to form nodules is initially greater for C-1 (Table 5). This proportion does not change under different cropping systems. Thus, estimates of either the total or effective populations may serve as an indicator of the other. In contrast, TA101 initially has a very low proportion of the total population which will form nodules. This changes in the presence of the host crop (100-fold increase) and for one of the non-host crops (wheat). This change may be due to selective pressure, where TA101 rhizobia with the ability to nodulate have an advantage. The high variability between total and effective TA101 populations means that estimates of either the total or effective populations will not serve as an indicator of the other.

The two isolates were different in competitiveness (Table 6). In a multi-strain inoculant C-1 occupied 100% of the nodules even though total numbers were not different.

Table 3. Change in the total rhizobial population in the root rhizosphere of host and non-host crops.

	TA101		C-1	
Crop[δ]	***[β]		*	
C.V.(%)	8.2		8.6	
Treatment	Log rhizobia g^{-1} soil			
Initial Count	5.3	cd[κ]	5.9	a
Pea	6.3	a	5.6	a
Soybean	6.0	ab	5.9	a
Wheat	5.6	bc	5.5	a
Flax	5.1	de	5.4	a
Fallow	4.5	e	4.7	b

δ - Log transformation.

β - *,*** - refer to $p < 0.05$ and $p < 0.001$, respectively.

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

Table 4. Change in the effective rhizobial population in the root rhizosphere of host and non-host crops.

Treatment	TA101		C-1	
	Log rhizobia g^{-1} soil			
Initial Count	1.4	c[δ]	5.0	ab
Following:				
Pea	4.0	a	5.2	a
Soybean	1.5	c	4.6	b
Wheat	2.7	b	4.0	c
Flax	1.4	c	4.1	c
Fallow	0.2	d	3.8	c

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

Table 5. The ratio of total and effective rhizobial population as affected by crop.

Treatment	TA101		C-1
		Total/Effective	
Initial Count	15000		18
Following:			
Pea	140		3
Soybean	19000		21
Wheat	340		23
Flax	10000		32
Fallow	44000		31

Table 6. Percent occupancy of TA101 and C-1 in root nodules from the field pea cropping treatments inoculated with TA101 and C-1.

TA101	C-1	Dual
0	99 (±0.7)$^{\delta}$	1 (±0.7)

δ - 95% confidence limit of a binomial proportion.

Conclusions

1. The host crop (field pea) increased both the total population and the proportion of the population with the potential to nodulate.

2. Non-host crops maintained the total population at the initial levels, but did not increase the proportion of the population with the potential to nodulate.

3. Total populations of TA101 and C-1 were similar, but the potential for C-1 to form nodules was greater than TA101.

4. TA101 was not a competitive isolate in the presence of C-1, occupying < 1 % of the nodules.

References

Kucey, R.M.N. and Hynes, M.F. 1989 Populations of *Rhizobium leguminosarum* biovars *phaseoli* and *viceae* in fields after bean or pea in rotation with nonlegumes. Can. J. Microbiol. 35:661-667.

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