Alberta Agricultural Research Institute and

Potash Phosphate Institute of Canada Matching Grant No. AARI92M429

Influence of Phosphorus Fertilizer on the Soil Quality of Historic Irrigated Rotation U

Written by

M. J. Clapperton¹

A. M. Johnston 2 , H. H. Janzen 1 , and J. M. Carefoot 1

1 Agriculture and Agri-Food Canada, Research Station, Box 3000, Main,

Lethbridge, Alberta T1J 4B1

2Agriculture and Agri-Food Canada, Research Station, Box 1240, Melfort, Saskatchewan S0E 1A0

Table of Contents

List of Figures
List of Tables
Abstract
Acknowledgements

1.0 Introduction	7
1.1 Objective	7
2.0 Research Plan	8
2.1 Site Description	8
2.2 Assessment of Arbuscular Mycorrhizal Fungi	9
2.3 Plant Analyses	10
2.4 Soil Analyses	10
3.0 Results	11
3.1 Soil Analysis	11
3.2 Crop yield and tissue nutrient concentration	11
3.3 Arbuscular Mycorrhizal Fungi	17
4.0 Discussion	23
5.0 References	24

List of Figures

Figure 1. Percent colonisation by arbuscular mycorrhizal fungi of soft white spring wheat roots from irrigated Rotation U in 1992 as affected by the previous crop and historical phosphorus treatment.	18
Figure 2. The estimated length of root of soft white spring wheat colonised by arbuscular mycorrhizal fungi and the total estimated root length per plant in 1992 as affected by the previous crops and historical P treatment.	19
Figure 3. The percent colonisation by arbuscular mycorrhizal fungi of the roots of barley var. Virden in 1993 in irrigated Rotation U as affected by historical P treatment and the previous crops.	20
Figure 4. Length of root colonised by AM fungi and total length of root per plant in barley var. Virden collected in the field at the 3-4 open leaf stage in 1993 as affected by previous crop and historical P treatment	21
Figure 5. The most probable number (MPN) of infective propagules of AM fungi per g dry weight of soil in Rotation U in 1992 on soft white spring wheat var. Fielder and in 1993 on barley var. Virden.	22

List of Tables

Table 1. Total soil phosphorus, potassium, nitrogen, carbon, carbonnitrogen ratio and inorganic carbon concentration response tohistorical P fertilization for Rotation U, May, 1992.	13
Table 2. Spring soil nutrient concentration response to historical P application in Rotation U, 1992 and 1993.	14
Table 3. Wheat and barley dry matter yield response to historical P application in Rotation U, 1992 and 1993.	15
Table 4. Seedling and harvest tissue nutrient concentration for wheat and barley samples from Rotation U.	16

Acknowledgements

We thank the Alberta Agricultural Research Institute and the Potash Phosphate Institute of Canada for jointly funding this project and, Carol Braat, Dave Miller, Linda Kremenik, Lorna Selinger, Lisa Kalischuk-Tymensen, Derrick Kanashiro, Pierre Tourliere, Darryl Nakonechny, and John Unrau for technical assistance.

Abstract

The ten-year Rotation U at the Lethbridge Research Station, initiated in 1911, is one of the oldest continuously irrigated rotations in North America. Phosphate fertilizer was applied three years out of ten to half of each of the plots in Rotation U making these plots ideal to study the long-term effects of phosphorus (P) fertilizer management on soil quality characteristics. A detailed evaluation of soil quality and nutrient supply in the fertilized and unfertilized portions of all the plots in Rotation U was performed. In addition, the abundance of arbuscular mycorrhizal (AM) fungi was assessed as influenced by both historical P treatment and previous crop (1991 crop) on soft white spring wheat (Triticum aestivum L. var. Fielder) in 1992 and barley (Hordeum vulgare L. var. Virden) in 1993.

An assessment of the inoculum density and colonisation by AM fungi in crop plants showed a consistently significant interaction between historical P application, colonisation of roots by AM fungi, and previous crops. Colonisation of soft white spring wheat by AM fungi was significantly reduced by historical P application, depending on the previous crop, with no apparent subsequent effect on overall yield of wheat 'silage'. The same was true for barley except that there was a more significant interaction between historical P application and the previous crop than for wheat.

In both 1992 and 1993 historical P application significantly increased pre-seeding soil P. Historical fertilizer P application continued to increase biomass yield and nutrient concentrations of N and P in both wheat silage and barley grain. While no recent fertilizer P treatment was available for comparison, the effects of historical P applications are persistent in maintaining the high-output or productivity of this low-input irrigated rotation, Rotation U.

1.0 Introduction

The ten-year Rotation U at the Lethbridge Research Station, initiated in 1911, is one of the oldest continuously irrigated rotations in North America. Phosphate fertilizer was applied three years out of ten to half of each of the plots in Rotation U since 1933 making these plots ideal to study the long-term effects of phosphorus (P) fertilizer management on soil quality characteristics. In our study we have evaluated the changes in soil nutrient levels and nutrient availability under a high output - low input irrigated crop rotation. An assessment of the inoculum density and colonisation of arbuscular mycorrhizal (AM) fungi were included in this study because of their known importance to plant uptake of nutrients of low soil mobility and availability such as phosphorus. We have also considered the effects of previous crops as well as historical P application on colonisation by AM fungi. Presently, we are unaware of research that has evaluated the influence of both historical phosphorus application and preceding crop on the abundance of both AM fungi in the soil and colonisation on the roots of cereals under irrigated field conditions.

1.1 Objective

The objective of our study was to study the influence of historical phosphorus application on soil quality and nutrient supply in a low input irrigated crop rotation.

2.0 Research Plan

2.1 Site Description

The ten-year Rotation U at the Lethbridge Research Station, initiated in 1911, is one of the oldest continuously irrigated rotations in North America. Phosphate fertilizer was applied three years out of ten to half of each of the plots in Rotation U making these plots ideal to study the long-term effects of fertilizer management on soil quality characteristics. Rotation U occupies ten 0.4 ha plots, and has consisted of three years of alfalfa, one year of each of wheat and sugar beets, followed by three more years of alfalfa and one year of each of barley and oats. Each plot has received 34 tonnes/ha of barnyard manure twice in each 10-year cycle, and 110 kg/ha of phosphate fertilizer (0-43-0 from 1933 to 1939 and 11-48-0 from 1938 to present) on half of each plot three years in ten.

An evaluation of one of the ten plots in 1984 revealed that since 1911 both soil N and organic matter have increased in that portion of the plot not receiving P fertilizer. In spite of the large amounts of nutrient removal in alfalfa, sugar beets, cereal grain and baled straw, this loss appears to be balanced by the manure additions and the N fixation of the legume alfalfa (Dubetz, 1983). Results showed that while soil P was found to have remained fairly constant since 1911, deficiencies have become evident in the unfertilized checks for alfalfa and row crops. While the barnyard manure applied to the entire experimental area was rich in P, this amendment alone has proven insufficient in maintaining plant nutrient requirements (Dubetz, 1983). Between 1933 and 1980 P fertilizer increased the yield of sugar beets by 19%, barley by 16%, and alfalfa by as much as 50% over the unfertilized check.

In 1991 Rotation U was modified to include two more rotations, one with an annual legume (faba bean silage) and the other with the row crop corn (silage). Each of the new rotations is replicated three times representing nine of the original ten plots.

Rotation 1: Alfalfa - Alfalfa - Wheat - Barley

Rotation 2: Faba bean - Wheat - Faba bean - Wheat - Barley

Rotation 3: Corn - Wheat - Corn- Wheat - Barley

(The underlined rotation-phase represents 1993 crop)

2.2 Assessment of Arbuscular Mycorrhizal Fungi

Individual plants were collected from the field plots when they were at the three-leaf stage of growth. Plants were removed with a hand-trowel and as much of the root system as possible was removed. Plants were put into plastic bags and stored in the cold room at 0.5° C for not more than three months. To assess colonisation by VAM fungi plants were removed from the plastic bag, and the root system was gently removed from the soil. As much soil as possible was shaken from the roots. Roots were then washed in cool tap water and the roots and shoots separated, dried at 80° C for 72 h, weighed, and weights recorded. Dry roots were then stained according to the method described by Phillips and Hayman (1970). Stained roots were mounted on microscope slides and assessed for VAM fungi using a modification (Zak and Parkinson, 1982) of the line-intercept technique (Newman, 1966). Field sampling followed the split-plot experimental design, data was analysed accordingly using a two-way analysis of variance for a split-plot design.

The inoculum density of AM fungi in the field plots was determined using the most probable number (MPN) technique (Clapperton and Reid,

1992). Soil from around and shaken free from the individual root samples was pooled according to the P treatment within each plot to be used in the MPN study. Two barley seeds were sown into each conetainer (Stuewe and Sons, USA), and subsequently thinned to one plant based on uniformity of size. All plants were harvested after 4 weeks of growth in the greenhouse. Plants were watered to saturation as required, and fertilized once a week with quarterstrength Hoagland's solution. At harvest, plants were processed in the same manner as described above. Stained roots were examined under the dissecting microscope and the root sample was scored for presence of AM infection and estimates of the most probable number of infective AM propagules were obtained. Spore counts from soil were not performed because MPN is considered to be a better estimate of the density of AM fungi and the potential for infectivity of the soil than spore counts alone. Estimates of the most probable number of AM fungi were statistically analysed using a twoway analysis of variance with the appropriate error term for split-plot experimental design. In all cases there were three replicates.

2.3 Plant Analyses

Plant biomass and Zadoks growth stage (Tottman, 1987) was assessed weekly as weather permitted. All above-ground plant material was harvested from two seed rows 1 m in length, and then dried and weighed to assess plant biomass. Seedling tissue concentrations of N, P, and K were analysed on plant samples taken at the Zadoks Growth Stage of 14-21. The same nutrient analyses were done on wheat silage samples at the end of 1992. Analyses for barley grain in 1993 were not available at the time this report was written.

2.4 Soil Analyses

Soil samples were collected in depth increments of 0-15, 15-30, and 30-60 cm (5 reps) were collected from the fertilized and unfertilized areas of all nine plots in Rotation U at harvest (total 270 samples). These sampling depths were selected as the experimental area has historically been plowed to a depth of 20 cm. The following analyses were performed on these soil samples: total N, P, K, C, organic carbon, nitrate-N, and available P (from bicarbonate extractable). Nitrogen mineralisation was performed on soil samples collected in 1992 when all the plots were being subjected to the same treatment (soft white wheat). This procedure provides an estimate of the soil capacity to supply N as a plant nutrient.

3.0 Results

3.1 Soil Analysis

During the two-year time period of our study the surface soil showed a positive effect of historical fertilizer P application (Table 1) with no response for K or N, indicating a uniform effect on soils from historical cropping practices. Soil P remained constant to a depth of 60 cm, while total K and N showed a progressive decline. No differences were recorded for total soil carbon, carbon:nitrogen ratio and organic carbon in response to historical P application.

Nitrogen mineralisation, during a 4 week incubation period, showed no significant response to P application (Table 2). Similarly, no response was obtained to pre-seeding nitrate-N in both 1992 and 1993. However, in both years historical P application significantly increased pre-seeding extractable soil P.

3.2 Crop yield and tissue nutrient concentration

In 1992 Fielder soft white spring wheat (*Triticum aestivum* L.) was grown until hail damaged the crop on August 2. There were no significant differences in yield at biomass sampling times in response to historical P application until August 10 at which time the wheat 'silage' was harvested (Table 3). The poor establishment of the wheat crop in the spring of 1992 is reflected in the high CV values recorded for the early season biomass samples. Rotation U suffered severe hail damage in 1992 (hence the high CV values, and the harvest of the crop as silage). In 1993, historical P application increased Virden barley (*Hordeum vulgare* L.) biomass over the unamended treatment on four biomass sampling dates (Table 3). Grain yield was also increased in response to historical P application (Table 3).

No difference was recorded in seedling (Zadoks Growth Stage 14-21) tissue N, P, or K concentration from P application in this study (Table 4). While both seedling tissue N and K concentrations were rated as high (N>3.0; K>3.0) for wheat and barley, P concentration was low to marginal (low<0.15; marginal 0.15 - 0.25). this reflects the very low soil P recorded at seeding (Table 2). While no significant correlation's were recorded between seedling tissue nutrient concentration and either wheat silage of barley grain yield, both crops showed a significant harvest biomass yield response (Table 3). In addition wheat silage showed significantly higher tissue N and P concentrations.

Table 1. Total soil phosphorus, potassium, nitrogen, carbon, carbon:nitrogen ratio and inorganic carbon concentration response to historical P fertilization for Rotation U, May, 1992.

Nutrient	Soil depth	-P Nutrient Cor	+P ncentration	CV
	(cm)	%		
Total phosphorus	0-15	0.053	0.055 *	3
	15-30	0.051	0.051	5
	30-60	0.051	0.053	5
Total potassium	0-15	0.551	0.548	4
•	15-30	0.543	0.536	5
	30-60	0.482	0.459	12
Total nitrogen	0-15	0.183	0.187	6
3	15-30	0.168	0.172	6
	30-60	0.116	0.114	8
Total carbon	0-15	2.215	2.180	7
	15-30	2.136	2.071	15
	30-60	2.432	2.711	25
Carbon:nitrogen	0-15	9.9	9.8	7
	15-30	9.7	9.2	7
	30-60	9.3	9.5	8
Organic carbon	0-15	1.809	1.841	6
3	15-30	1.634	1.677	7
	30-60	1.076	1.080	8

^{*} Significantly different at P=0.05.

Table 2. Spring soil nutrient concentration response to historical P application in Rotation U, 1992 and 1993.

Sample	Depth (cm)	-P Nutrient concentra	+P ation	CV
1992	mg kg ⁻¹			
Nmin†	0-15	42.0	45.1	15
•	15-30	41.4	42.7	15
	30-60	24.3	23.9	16
Nitrate-N	0-15	47.7	51.5	29
	15-30	43.4	43.7	17
	30-60	33.6	33.7	24
Phosphorus 0.05 M Na HCO ₃ e	0-15 xtractable P	0.96	1.62 +	52
1993				
Nitrate-N	0-15	15.8	16.3	25
	15-30	19.1	20.4	28
	30-60	16.9	17.2	41
Phosphorus	0-15	1.58	2.25 +	40

^{+,*,**} Significant at P=0.10, 0.05 and 0.01, respectively.
† Nmin= N mineralisation in a 4 week laboratory incubation under favourable conditions

Table 3. Wheat and barley dry matter yield response to historical P application in Rotation U, 1992 and 1993.

Sample	-P	+P	CV	
	kg ha	1		
May 28	32	36	40	
June 4	101	111	32	
June 11	270	301	26	
June 18	500	515	35	•
June 25	1380	1307	23	
July 13	3951	4203	$\frac{17}{17}$	*
August 10 (harvest)	6195	7599 +	21	
1993 Barley				: :
May 11	49	50	14	
May 18	124	148 +	20	
May 25	$\frac{1}{374}$	492 *	$\frac{1}{21}$	
June 9	1810	2369 **	18	
June 18	3004	3362	17	
August 24	10703	11812 *	9	
Grain Yield	4358	4917 **	7	

^{+,*,**} Significant at P=0.10, 0.05 and 0.01, respectively.

Table 4. Seedling and harvest tissue nutrient concentration for wheat and barley samples from Rotation U.

Sample		-P Nutrient concentr	+P ation	CV
1992 Wheat		%		
Seedling	N	4.04	3.90	11
	P	0.19	0.20	21
	K	3.06	2.96	5
TT (11)	N T			
Harvest (silage)	N	0.86	1.03 +	20
	P	0.09	0.13 **	25
	K	1.43	1.31	18
1993 Barley				
Seedling	N	4.67	4.91	6
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	P	0.14	0.15	4
	K	3.46	3.38	4
	17	0.10	0.00	<del>-1</del>

^{+,*,**} Significant at P=0.10, 0.05 and 0.01, respectively.

### 3.3 Arbuscular Mycorrhizal Fungi

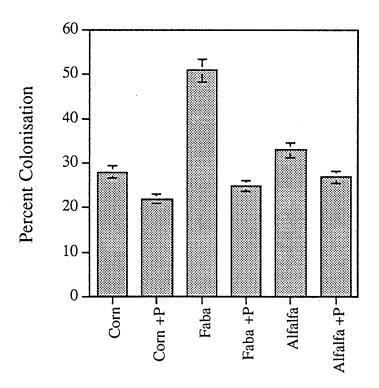
Assessment of the inoculum density and colonisation by AM fungi in seedlings showed a consistently significant interaction between historical P application, and colonisation of roots by AM fungi, depending on the previous crop. In 1992, percent colonisation by AM fungi was consistently and significantly lower in soft white spring wheat var. Fielder in response to historical P application (Figure 1). A significant (a=0.05) interaction between percent colonisation, historical P application, and previous crop was indicated after two-way ANOVA. The same pattern of colonisation in response to fertiliser application is shown in estimates of total root length colonised by AM fungi and total root length (Figure 2).

In 1993, there was a completely different pattern of AM colonisation (Figures 3 & 4). The percent AM colonisation of barley grown in plots that had been previously (1991) cropped in corn and then soft white spring wheat in 1992 showed a significant decrease with historical P application (Figure 4). Whereas, barley grown in plots that had been previously cropped to legumes showed a significant increase in percent colonisation in response to historical P application (Figure 4). There was a significant (a=0.01) effect of previous crop and interaction between previous crop and historical P application in terms of percent and total root length colonised by AM fungi (Figures 3 & 4).

The assessment of inoculum density of AM fungi (Figure 5) shows that the pattern of inoculum density is closely related to the pattern of colonisation for soft white spring wheat and only similar to that of barley. The results of the assessment of AM inoculum density with respect to historical P application clearly show that the previous crop also strongly affects the infectivity of AM fungi in the subsequent crop (Figure 5). However, we assessed the inoculum density of soft white spring wheat and barley at the

same time using soil from the spring of 1993 after being cropped to wheat and before barley. We assessed inoculum density of AM fungi in soil samples taken in the spring because we have previously determined that spring soils have the highest inoculum densities, giving more accurate results. Therefore, the results of the MPN to determine the inoculum density of AM fungi after barley were unavailable at the time this report was written.

**Figure 1.** Percent colonisation by arbuscular mycorrhizal fungi of soft white spring wheat roots from irrigated Rotation U in 1992 as affected by the previous crop and historical phosphorus treatment.



Previous Crop and P Amendment

Figure 2. The estimated length of root of soft white spring wheat colonised by arbuscular mycorrhizal fungi and the total estimated root length per plant in 1992 as affected by the previous crops and historical P treatment.

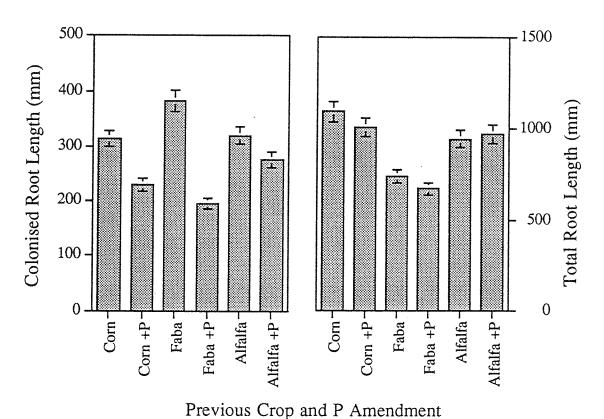
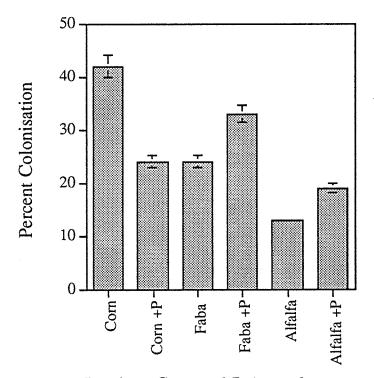


Figure 3. The percent colonisation by arbuscular mycorrhizal fungi of the roots of barley var. Virden in 1993 in irrigated Rotation U as affected by historical P treatment and the previous crops.



**Figure 4.** Length of root colonised by AM fungi and total length of root per plant in barley var. Virden collected in the field at the 3-4 open leaf stage in 1993 as affected by previous crop and historical P treatment.

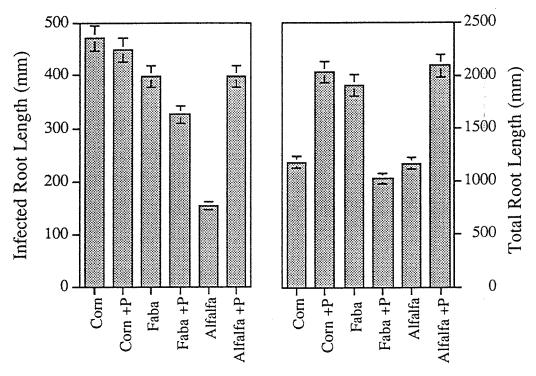
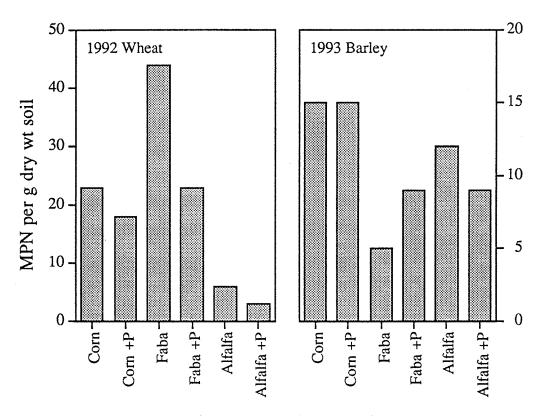


Figure 5. The most probable number (MPN) of infective propagules of AM fungi per g dry weight of soil in Rotation U in 1992 on soft white spring wheat var. Fielder and in 1993 on barley var. Virden.



Previous crop and P amendment

### 4.0 Discussion

Colonisation of soft white spring wheat by AM fungi was significantly reduced by historical P application, depending on the previous crop, with no apparent subsequent effect on overall yield of wheat 'silage' yield. The same was true for barley except that there was a more significant interaction between historical P application and the previous crop than for wheat. The negative interaction between fertiliser P and AM fungi is well documented in the literature, although not for such low concentrations of P. The fact that yield was not affected by the significantly reduced colonisation by AM fungi supports the hypothesis that the mycorrhizal dependence of the native races has been removed from cereal grasses through breeding programs (Hetrick, Bockus, and Bloom, 1984; Boyetchko and Tewari, in press). However, there were no differences in the seedling nutrient concentrations of wheat and barley with respect to P application despite the higher concentration available P at pre-seeding. This suggests the possibility that plants grown in soil without historical P application are benefiting from increased colonisation by AM fungi during seedling establishment.

The inoculum density of AM fungi follows the same pattern of variation with respect to historical P application and previous crops as the root colonisation for wheat. After wheat the inoculum density of soil for barley was much lower indicating a significant effect of previous crops on fungal communities. Continued research into the effects of soil interactions including the effects of previous crops is warranted.

In summary, low spring soil P levels resulted in P deficient seedlings for both wheat in 1992 and barley in 1993. However, historical fertiliser application continues to significantly increase wheat silage yield and N and P concentrations, and barley grain yield. Colonisation by AM fungi was

significantly reduced by historical P application depending on the previous crop. However, this had no apparent effect on biomass or crop yield in 1992 or 1993. While no recent fertiliser P treatment was available for comparison, the effect of historical P applications are persistent and continue to be recorded in current crop yield and quality characteristics.

## 5.0 References

- Clapperton, M. J. and Reid, D. M. 1992. A relationship between plant growth and increasing VA mycorrhizal inoculum density. New Phytologist 120: 227-234.
- Dubetz, S. 1983. Ten-year irrigated rotation U. 1911-1980. Research Branch, Agriculture Canada.
- Hetrick, B. A. D., Bockus, W. W., and Bloom, J. 1984. The role of vesicular-arbuscular mycorrhizal fungi in the growth of Kansas winter wheat.

  Canadian Journal of Botany 62: 735-740.
- Newman, E. I. 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology, 3: 139-145.
- Phillips, J. M. and Hayman, 1970. Improved procedures for cleaning roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55: 158-161.
- Tottman, D. R. 1987. The decimal code for the growth stages of cereals, with illustrations. Ann. Appl. Biol., 110 441-454.
- Zak, J. C. and Parkinson, D. 1982. Initial vesicular-arbuscular mycorrhizal development of slender wheat grass on two amended mine spoils. Canadian Journal of Botany, 60: 2241-2248.