Environmental Factors Influencing Numbers of *Rhizobium leguminosarum* biovar *trifolii* and Its Bacteriophages in Two Field Soils

**KERRIE A. LAWSON,**1 **YVONNE M. BARNET,**1* and **CLYDE A. McGILCHRIST**2

Schools of Microbiology1 and Mathematics,2 The University of New South Wales, Kensington, New South Wales 2033, Australia

Received 3 December 1985/Accepted 25 August 1986

Fluctuations in numbers of *Rhizobium leguminosarum* biovar *trifolii* and its bacteriophages in two fields with different soil types were followed during a 17-month period in 1981 and 1982. Mean levels of both phage and rhizobial varied significantly (*P < 0.05*) on different occasions, with rhizobial levels varying from $1.6 \times 10^2$ to $2.0 \times 10^4$ cell per g of soil and phage from 0 to $1.7 \times 10^4$ PFU/g of soil. Multivariate regression analysis showed rhizobial levels to be significantly and positively related to vegetation height and solar radiation, but not to mean temperature, precipitation, soil matric potential, or soil type. Rhizobiophage concentrations were significantly and positively related to soil matric potential and vegetation height. They were reduced in the silty clay loam soil, although the presence of 34% clay did not prevent phage multiplication and the occurrence of high phage levels.

It has been observed that *Rhizobium* inoculant strains frequently fail to persist in soil and cannot be recovered, at least in their original genetic form, relatively soon after inoculation (16). Even when they do survive, they may be unsuccessful in nodule formation in the field situation (e.g., see reference 42). Although environmental factors are presumably responsible for these effects, knowledge of *Rhizobium* ecology is so limited that we can usually offer no explanation for the observed phenomena. Much more information on the dynamics and factors influencing rhizobial populations in soils is needed before we can understand what is happening at individual sites and more efficiently manage the *Rhizobium*-legume symbiosis.

The action of bacteriophages is one factor frequently proposed as a mechanism of change in bacterial populations. Phages have the ability to limit host numbers in a selective fashion as well as to act as vehicles for genetic exchange, and it has been suggested that they may be of general importance in bacterial evolution over both the short and the long term (36, 39). Rhizobiophages have been isolated from soils and nodules on many occasions (2, 26, 28, 46). In vitro experiments have shown that they may influence both the size and the nature of populations of their host. They can affect the outcome of competition for nodulation between rhizobial strains and may select for phage-resistant bacterial variants (4, 13). Since acquisition of phage resistance is often associated with a loss of infectivity and symbiotic effectiveness (3, 20, 25, 35), similar selective activity in soil could decrease the overall nitrogen-fixing potential of a field population of root-nodule bacteria. In addition, transduction between rhizobia has been described many times (27, 32) and has the potential to change the characteristics of inoculum strains in soil.

It is not known if any of these effects occur in natural habitats. Interactions in the soil may be similar to those seen in laboratory experiments, but it is also possible that phage activity is limited in this complex and heterogeneous environment. We need much more data on factors influencing phage activity and on phage levels and growth rates in natural habitats before it will be possible to assess their effects on populations of rhizobia and other bacteria in soil. Only a few studies have attempted to obtain such information (10, 11, 17, 28), and details of the edaphic and climatic factors which affect rhizobiophage population dynamics are unavailable.

This study was undertaken to monitor the numbers and distribution of *Rhizobium trifolii* and its phages in legume pastures and to assess the importance of a range of environmental factors as determinants of phage and rhizobial population levels.

**MATERIALS AND METHODS**

**Sites and sampling.** The sampling sites were situated at Camden, on the eastern coastal plain of New South Wales, Australia (latitude 34°03'S, longitude 150°42'E). They consisted of two neighboring areas of pasture at the research farms, Lansdowne and Corstorphone, operated by the University of Sydney. The vegetation of the two areas consisted predominantly of the introduced pasture grasses, *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu grass) and *Paspalum dilatatum* Poir. (paspalum), and the pasture legumes *Trifolium repens* L. (white clover), *Trifolium subterraneum* L. (subterranean clover), and *Trifolium fragiferum* L. (strawberry clover). The soils at the two sites were classified as a sandy loam and a silty clay loam on the basis of particle size distribution (31). More detailed characteristics are summarized in Table 1. Particle size distribution was determined by the hydrometer and sieving method modified from Day (9), using the criteria of the International Society of Soil Science. Soil pH was determined for 10-g soil samples suspended in 20 ml of 0.01 M CaCl₂, using an Ionode non-flow pH electrode and an LC-80 pH-mV meter (TPS Pty. Ltd., Brisbane, Australia) (37). The soil moisture characteristic was determined by the method of Richards (40),

---

1 Corresponding author.
TABLE 1. Characteristics of two field soils

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Soil classification</th>
<th>pH</th>
<th>Gravel &lt;2 mm</th>
<th>Coarse sand 0.18–2 mm</th>
<th>Fine sand 0.02–0.18 mm</th>
<th>Silt 0.002–0.02 mm</th>
<th>Clay &lt;0.002 mm</th>
<th>Water content (g/g of dry soil) at matric potential (bars):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landsdowne</td>
<td>Sandy loam</td>
<td>5.55 ± 0.08</td>
<td>0.8 ± 0.5</td>
<td>56.6 ± 4.0</td>
<td>18.3 ± 1.1</td>
<td>12.0 ± 1.3</td>
<td>11.3 ± 1.0</td>
<td>0.154 ± 0.099 ± 0.086 ± 0.073 ± 0.015 ± 0.010 ± 0.007 ± 0.005 ± 0.413 ± 0.351 ± 0.297 ± 0.243 ± 0.017 ± 0.026 ± 0.025 ± 0.007</td>
</tr>
<tr>
<td>Corstorphine</td>
<td>Silty clay loam</td>
<td>4.85 ± 0.04</td>
<td>0.4 ± 0.4</td>
<td>10.5 ± 1.8</td>
<td>12.9 ± 0.7</td>
<td>42.8 ± 1.6</td>
<td>34.4 ± 1.8</td>
<td>0.154 ± 0.099 ± 0.086 ± 0.073 ± 0.015 ± 0.010 ± 0.007 ± 0.005 ± 0.413 ± 0.351 ± 0.297 ± 0.243 ± 0.017 ± 0.026 ± 0.025 ± 0.007</td>
</tr>
</tbody>
</table>

* Results are reported as the mean ± 95% confidence interval.

** ISSS, International Society of Soil Science.

using 5- and 15-bar ceramic plate extractors (Soil Moisture Equipment Co., Santa Barbara, Calif.).

Soil samples were taken from four 1-m² plots which were permanently fixed at 25-m intervals in each field and protected from grazing by electric fences. Sampling took place at approximately monthly intervals during a period of 17 months between June 1981 and November 1982. On each occasion two 10-cm soil cores were taken at random from each of the eight plots. Soils were stored, when necessary, at 4°C before assessment.

**Rhzobial and phage strains.** Bacterial nomenclature used throughout this paper is that proposed by Jordan (24). With the exception of *R. leguminosarum* biovar trifolii strains NU341 and NU342, which were isolated during this study from nodules collected at Lansdowne and Corstorphine, respectively, all bacteria and phage used were obtained from collections maintained at the University of New South Wales.

**Rhzobial enumeration.** Soil samples for bacterial and phage enumeration were homogenized (three 2-s bursts) in a Waring blender. A 10-g subsample was then shaken with 100 ml of distilled water at 200 oscillations per min for 15 min in a DynaMAX wrist-action shaker (Ainsworth Dental Co., Sydney, Australia) and allowed to settle. The supernatant was serially diluted 10-fold, and 1-ml samples were inoculated onto duplicate 1-week-old *T. repens* seedlings grown in tube culture on Jensen’s seedling agar (23). Nodulation of test plants was checked 6 weeks after inoculation. Rhizobial levels were determined by the plant infection method, using the modified most-probable-number tables of Fisher and Yates (48).

**Rhzobiophage enumeration.** A 10-g homogenized soil sample was shaken with 20 ml of diluent in a reciprocal shaker for 40 min at 26°C and allowed to settle. The decanted supernatant was either centrifuged at 1,475 × g in a bench centrifuge (MSE) or shaken in a wrist-action shaker (200 oscillations per min) with 20% chloroform for 15 min. One milliliter of the supernatant and 1 ml of a 10-fold dilution were plated in soft yeast succrose agar (6) with 0.5 ml of an 18-h culture of the host indicator strain. Plaque formation was assessed after 2 to 4 days of incubation at 26°C. For assessment of the method, suspensions of rhizobiophages NT1 and CT6 (2) containing approximately 10³ PFU/g of soil were inoculated into soil, and their elution and recovery were determined. Initial soil assessment used as plaque indicators nine *R. leguminosarum* biovar trifolii strains representing a wide range of serotypes and of phage sensitivity patterns, SU36, SU61, SU91, SU94L, SU94S, SU157, SU204, SU297/31, and SU329 (2), and two field isolates, NU341 and NU342. Strains SU91, NU341, and NU342 were adopted for subsequent titrations.

**Soil matric potential.** The gravimetric moisture content of duplicate 10-g subsamples of each soil sample was determined after drying at 105°C for 24 h and converted to matric potential by using a calibration graph of the moisture characteristic of the appropriate soil. An individual moisture characteristic was used for plot 3 at Lansdowne as its moisture relations differed significantly (*P* ≤ 0.05) from those of the other three Lansdowne plots after analysis of variance. For the remaining Lansdowne and Corstorphine plots, data were pooled to provide the respective moisture characteristics.

**Climatic data.** The maximum and minimum daily air temperatures, cumulative solar radiation, and cumulative rainfall were obtained from the records of the university weather station at Camden. Rainfall records were augmented after March 1982 by rain gauge measurements, using Marquis “1000” rain gauges (Commonwealth Moulding Pty. Ltd.) in the individual fields. Soil temperatures in each field were determined after February 1982, using continuous recording thermographs (Sato Instruments).

**Vegetation monitoring.** The height of vegetation was measured in each plot. The dominance of clover or grass in each plot was evaluated subjectively, and the flowering periods of clover plants were noted.

**Statistical analyses.** Rhizobial and rhizobiophage concentrations were transformed logarithmically for statistical analyses. The effect of plot and sampling occasion on both rhizobial and rhizobiophage levels was tested by two-way analysis of variance with replication. The Student-Newman-Keuls procedure (47) was used to determine which pairs of population means differed significantly from each other. The significance of the relationship between the two independent variables, rhizobial and rhizobiophage levels, was determined with the correlation coefficient. This was transformed to a *z* value, which was compared to a standardized normal variate (47).

A multivariate regression analysis (33) based on a linear additive model was used to determine the relationship between the dependent variables, rhizobial and rhizobiophage levels, and a range of independent environmental variables. Except in the case of the climatic variables, the analysis used values for individual plots or soil samples.

**Models used were of the form**

\[ Y_{1,t} = \alpha_0 + \alpha_1 Y_{1,t-1} + \alpha_2 Y_{2,t-1} + \alpha_3 X_1 + \ldots + \alpha_n + \varepsilon X_n \]

\[ Y_{2,t} = \beta_0 + \beta_1 Y_{1,t-1} + \beta_2 Y_{2,t-1} + \beta_3 X_1 + \ldots + \beta_n + \varepsilon X_n \]

where *Y*₁,ₜ = rhizobial level on sampling occasion *t*; *Y*₂,ₜ = rhizobiophage level on sampling occasion *t*; *X*ₙ = environmental variable *n*; and *α* and *β* = partial regression coefficients.
The partial regression coefficient for each independent variable was determined by the least-squares procedure. The significance of each partial regression coefficient was determined by a $t$ test.

To determine the stability or otherwise of the partial regression coefficients, their values were estimated recursively and graphed. The recursive estimates are made with data available up to each particular time point.

Independent variables found not to be significantly associated with either rhizobiophage or rhizobial levels were progressively eliminated from the analysis. The Wilks-Lambda Criterion was used to test the validity of these eliminations. When independent variables were found to be related, as in the case of mean weekly and monthly air and soil temperatures, then a single representative variable only was used in the analysis.

The possibility of a parabolic, rather than a linear, relationship between the rhizobiophage and rhizobial levels and matric potential was tested, using the square of the matric potential term as an independent variable in the analysis. Similarly, interaction between matric potential and soil type was tested by including the product of those terms as an independent variable. As the effect of additional clay content in the silty clay loam soil was of primary interest, soil type was included as either 0 (sandy loam at Lansdowne) or 1 (silty clay loam at Corstorphine).

RESULTS

Development of methods for phage enumeration. The most satisfactory method for enumerating phage from soil was determined by experiments in which phages CT6 and NT1 were added to, and then recovered from, samples of the two soil types. Yeast sucrose broth proved to be the most effective diluent for phage enumeration, producing higher counts than distilled water and nutrient broth at pH 7.2 and 8.0. Bacteria were removed more effectively, and higher phage counts were maintained, when the lysate was centrifuged, rather than shaken, in the presence of chloroform. Elution in yeast sucrose broth followed by centrifugation into chloroform was thus adopted for phage enumeration from soils. This method was effective in recovering between

70 and 80% of phage CT6 from both soils. Recovery of phage NT1 was of the same order but was more variable and depended upon the soil type, being less from the sandy loam soil.

Initial assessment of rhizobial strains for ability to act as hosts for phages present in the field soils revealed that only three laboratory strains, $R. \text{leguminosarum}$ SU36, SU61, and SU91, and the two field isolates NU341 and NU342 supported plaque formation. All positive samples produced plaques on indicator strain SU91, while strains SU36 and SU61 had narrower plaque susceptibility ranges and showed plaques from only a proportion of positive samples. All further titrations were performed with $R. \text{leguminosarum}$ SU91, NU341, and NU342. As the same phages can produce plaques on different host strains, phage numbers recorded were the highest count on a single indicator strain and not the sum of all plaques seen.

Monitoring of rhizobial and rhizobiophage levels. $R. \text{leguminosarum}$ biovar trifolii was detected in all soil samples on every sampling occasion. Fluctuations in rhizobial field populations are shown in Fig. 1. Mean levels varied from $2.1 \times 10^2$ to $1.4 \times 10^4$ cells per g of soil at Lansdowne and $1.6 \times 10^2$ to $2.0 \times 10^4$ cells per g of soil at Corstorphine. The maximum level found in an individual soil sample was $8.4 \times 10^5$ cells per g of soil. Although there was considerable variability in the levels of rhizobia detected in individual soil samples on a single occasion, a significant difference ($P < 0.05$) was observed between mean levels on different occasions in both fields, indicating that the rhizobial populations were not static. Differences between mean levels in different plots were not significant ($P < 0.05$) at Corstorphine, but were at Lansdowne, where plot 3 contained higher rhizobial numbers than adjacent plots. This plot also showed consistent differences in plant growth and soil moisture.

Rhizobiophages were detected in each plot on a number of occasions. A total of 77% of sandy loam samples and 56% of silty clay loam samples yielded rhizobiophages able to produce plaques on at least one of the host strains. Fluctuations in mean rhizobiophage levels are shown in Fig. 2. Mean phage levels in different plots varied significantly ($P < 0.01$) at both sites, implying that the phages were not uniformly distributed throughout the fields. Significant dif-
ferences ($P < 0.01$) were also observed between mean levels on different occasions, showing that phage population sizes varied with time.

Rhizobial and rhizobiophage levels were positively and significantly correlated ($P < 0.01$). There was no evidence of an inverse relationship between the sizes of the two populations.

A range of plaque types was observed. They were clear, turbid, and "bullseye," as described by Barnet (2), and varied in size from pinpoint to 5 mm in diameter. A single soil sample often yielded a variety of plaque types, and the types derived from a particular plot also varied on different occasions. Plaque counts produced by any soil sample differed according to the rhizobial strain used as host indicator. Levels observed with different hosts during a typical 4-month period are shown in Table 2. In most cases the greatest number of plaques was observed with strain SU91. Plaque numbers with the two field isolates often followed a similar pattern of fluctuations, although relative numbers differed between sampling occasions and between the two fields. Strain NU341 (originally isolated from Lansdowne) was much less sensitive than either SU91 or NU342 (from Corstorphine) to phages from Corstorphine.

Monitoring of environmental variables. The fluctuations in climatic and other environmental variables are presented in Fig. 3. As expected, weekly and monthly air and soil temperatures, and total weekly solar radiation, followed seasonal patterns, reaching maximum values during the period December 1981 to February 1982 (Australian summer) and minimum values between July and August (Australian winter). The vegetation height in each field was also related to season, increasing rapidly in summer and autumn and declining during winter when the vegetation also appeared dry and brown. Clover height did not necessarily reflect the height of total vegetation. Clovers predominated in the pasture in spring of each year, but were otherwise overshadowed by the grasses, and were in flower at sampling times during September 1981 to January 1982 and after October 1982.

Monthly precipitation, which included sprayed irrigation water, was irregular and lower than normal, reflecting the drought conditions prevailing in eastern Australia during the first months of the survey. Soil matric potential was also very variable, differing within and between plots and between occasions. It varied from levels drier than the conventional permanent wilting point of plants (−15 bars) to wetter than field capacity, particularly in the silty clay loam at Corstorphine. When waterlogged, this soil occasionally appeared deflocculated. Soil pH (Table 1) was found not to vary significantly ($P = 0.05$) either within a field or during the course of the survey.

**Relationship between population levels and environmental variables.** Multivariate regression analysis yielded partial regression coefficients relating rhizobial and rhizobiophage levels to environmental variables. Mean temperature and rainfall were found not to be significant determinants of either rhizobial or rhizobiophage levels and the fit of the regression equations, as indicated by the Wilks-Lambda criterion, was significantly affected when these variables were eliminated. The environmental variables found to be significantly related to either rhizobial or rhizobiophage levels are listed in Table 3, together with their partial regression coefficients and levels of significance. Partial regression coefficients were estimated recursively and plotted (not shown). Their values based on the initial data were not appreciably altered by inclusion of later data, thus indicating that they were reliable estimates.

The final regression equations describing rhizobial and rhizobiophage levels were:

\[
\ln (1 + Y_{1, t}) = 3.36 - 0.884 X_1 + 2.65 X_2 - 1.33X_3 + 0.778 X_1 \cdot X_3
\]

\[
\ln (1 + Y_{2, t}) = 3.17 + 0.321 \ln (1 + Y_{2, t - 1}) + 3.50 X_2 + 0.0167 X_4
\]

where $Y_{1, t} =$ rhizobiophage concentration on sampling occasion $t$ (PFU per gram of soil); $Y_{2, t} =$ rhizobial concentration on sampling occasion $t$ (cells per gram of soil); $X_1 =$ log (−matric potential [bars]); $X_2 =$ vegetation height (meters); $X_3 =$ soil type (0 represents sandy loam soil, 1 represents silty clay loam soil); and $X_4 =$ solar radiation (megajoules per square meter).

Rhizobiophage levels were significantly ($P < 0.05$) and positively related to the logarithm of negative soil matric potential, but this relationship was confined to the sandy loam soil. In the silty clay loam the inclusion of the interaction term, log (−matric potential) × soil type, which was also found to be significant, effectively negated the relationship. Thus, matric potential accounted for a large proportion of the variation in rhizobiophage levels in the sandy loam, but was of negligible importance in the silty clay loam. The lack of significance of the square of the log (−matric potential) term, which increases at both extremes of moisture content, indicated that the relationship between matric potential and rhizobiophage levels was not parabolic in either soil. Rhizobiophage levels were also significantly and positively related to vegetation height and reduced in the silty clay loam soil.

Rhizobial levels were significantly related to vegetation height and cumulative weekly solar radiation, but not to any of the soil moisture, soil type, or temperature and rainfall terms.

After the conclusion of the main part of the study, an attempt was made to check the predictive value of the derived regression equations. The significant environmental variables were measured in February 1983, and the equations were used to calculate expected values for the size of phage populations. The actual population was within 10% of the predicted value.

**DISCUSSION**

Rhizobial population levels at Camden were comparable to those previously reported in clover pastures of southeast-
ern Australia (12, 15, 42). Rhizobiophage numbers were considerably greater than those reported by some authors (1, 38) but comparable to the levels found by others (10, 11, 28). There were indications from the variations in both plaque type and the proportion of total phage numbers to which different hosts were sensitive that the composition of the phage population varied between locations and with time. Our values are minimum estimates of the size and diversity of phage populations since the bacterial indicators are selective in their sensitivity and actual phage numbers were probably even greater than our measurements indicate. It is also probable that, since these values were derived from bulked soil samples, higher concentrations existed at individual microsites.

The levels detected raise the possibility that phages may have effects on their host populations. Although positive correlation of phage and bacterial numbers indicates that the viruses have no significant role in reducing host population levels, more subtle interactions, such as the selection of phage-resistant variants or the promotion of genetic exchange, remain possibilities.

Associations were found between environmental variables and both rhizobial and rhizobiophage population sizes. Rhizobial levels were significantly and positively related only to solar radiation and vegetation height. This is consistent with the observation that, in natural habitats, carbon and energy availability are the main determinants of bacterial population size (e.g., see reference 45). In soil, exudates from plant roots are a major source of nutrients for bacterial growth (8). Since the quantity of exudate is directly related...
to photosynthetic activity (8), it is not surprising that factors such as plant size and solar radiation, which influence the rate of photosynthesis, should show significant association with rhizobial numbers. The failure to observe correlation of bacterial population size with other environmental variables (temperature, precipitation, and soil matric potential) is consistent with the observations of recent workers (30, 34), although contrary to some (14, 44), but not all (22), reports in the older literature. The relationship with vegetation height explains the higher rhizobial concentrations found in plot 3 at Lansdowne, which also supported greater plant growth than adjacent plots.

The effect of environmental factors on phage populations seem more likely to have been due to changes in phage multiplication and survival than to altered release rates from lysogens. Large-scale prophage induction is a consequence of conditions which damage DNA or inhibit DNA replication (29). There is no reason to believe that the environmental factors implicated in this study produce such conditions.

The observed effects of environment on bacteriophage populations are explained best by the thesis that they operate indirectly through effects on the density or accessibility of host bacteria. Positive correlation between host and phage numbers as well as the axiomatic host dependence of viral multiplication support this suggestion. Bacterial microcolonies occupy only a small proportion of available surfaces in soil (18, 43). For continued growth, phage must be able to reach new hosts which may be some distance away. The rate at which transfer will occur will depend on both the concentration of bacterial cells and phage mobility. Higher rhizobial levels, such as those associated with higher vegetation biomass, would be expected to produce increases in phage numbers, an effect seen in this study. Significant correlations of phage levels with both soil water potential and soil type were also observed. These environmental parameters seem most likely to produce their effects through their capacity to cause changes in phage diffusion rates. The movement of bacteria has been shown to increase at soil water levels which produce a greater continuity of water-filled pathways (19, 21), and it seems likely that phage diffusion would be affected similarly. High clay contents would also be expected to affect phage mobility. Clay colloids can both adsorb phages (7) and protect sensitive bacteria from attack by blocking surface structures (41). These effects do not necessarily limit equilibrium phage numbers in homogeneous environments (e.g., chemostat vessels [5]), but could do so in the soil in which clays are usually associated with large aggregates. Adsorption to aggregates in high-clay soils such as those at Corstorphine would reduce phage mobility. This would account for both the lower average phage levels and the lack of response to increasing matric potential at this site, since phage particles would have reduced ability to reach new hosts even at high water contents. However, even at this clay concentration (34%) phage multiplication was not completely prevented, and when other factors were favorable (as occurred when the environment was supportive of increased host numbers), phage levels as high as those in the low-clay soil were reached.

ACKNOWLEDGMENTS

Our thanks are extended to the Departments of Agronomy and Animal Husbandry, University of Sydney, for permission to use the field sites and to Gay Booth and George Novak for provision of climatic records. Appreciation is also expressed to M. Melville for expert advice on soil analysis and to Peter Catt for helpful suggestions during preparation of this manuscript.

This work was supported in part by a grant from the Australian Research Grants Scheme. We also acknowledge the Commonwealth Postgraduate Research Award held by K.A.L.

LITERATURE CITED


TABLE 3. Relationship of rhizobium and rhizobiophage concentrations to environmental variablesa

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>Standardized partial regression coefficientb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhizobiophage concn</td>
</tr>
<tr>
<td>Matric potential (log [bars])</td>
<td>-0.386**</td>
</tr>
<tr>
<td>Vegetation ht (m)</td>
<td>0.142*</td>
</tr>
</tbody>
</table>
| Cumulative weekly solar radia-
| tion (MJ m⁻²)                 | -0.0461 NS         | 0.268**         |
| Soil type (0 = sandy loam; 1 =
| silty clay loam)              | -0.239**           | 0.0271 NS       |

a Rhizobiobium and rhizobiophage concentrations were included in regression equations as their natural logarithms.

b NS: Not significant; *significant (P < 0.05); **significant (P < 0.01).


