
Perpetuation pattern and population dynamics of *Ralstonia marginalis* causing marginal leaf spot of rice and barley

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The spread of marginal leaf spot of rice and barley in field took place through high inoculum build up of *Ralstonia marginalis* in lesioned leaves and stems. The bacterial population in these lesions increased parallel to maturation of the crop. Leaf lesions had higher bacterial numbers than those of the stem. This was true for each of the three rice (IR-36, Mussorie, Rajendra 202) and barley (Karan 4, BAU 2005, BR-32) cultivars separately investigated. Lesions of rice cv. IR-36 and barley cv. Karan 4 had highest bacterial numbers in comparison to other cvs. Survival of the pathogen significantly occurred in the soil; more at depth level of 2.5 cm than at 7.5 cm. The bacterial population in the soil too increased progressively upto late of September; which then declined till middle of November. With the harvest of the rice crop there was further diminution in bacterial numbers in the fallow soil. Even with declining numbers till early January the pathogen survived in the soil. However, in November inoculum build up takes place concurrently in rice straw lying in the field even after harvest. With sowing of barley in January and its maturation in March the bacterial population increased in the soil, and in stem and leaf habitats of standing barley crop. The pathogenic inoculum maintained in rice tissues earlier and sustained in soil and straw continued to survive further in the soil, where barley was sown and was maturing. From late March onwards the diminution in bacterial population in the soil continued till harvest of barley, and after that in the fallow soil up to May and late June. The fallow soil, after harvest of barley, and also the straw of barley, both were instrumental in sustaining bacterial inoculum till rice seedlings were transplanted. It is apparent that perpetuation of the disease takes place through surviving bacteria in the soil, and in straw of both the cereals. A continued alternate cropping of rice and barley in the same field helped in sustained inoculum presence and carry over of the disease from year to year.

Key words : *Ralstonia marginalis*, rice, barley, soil, survival, disease-perpetuation, inoculum, buildup bacterial population

INTRODUCTION

Ecology of phytopathogenic bacteria and its role in perpetuation of disease has been emphasized for different bacterial diseases, like soft rot and black leg of potato caused by *Erwinia carotovora* subsp. *carotovora* and *atroseptica* (Perombelom, 1980; Perombelom and Kelman, 1980). However, phytopathogenic *Corynebacterium* sp., in contrast to the soft rot *Erwinia* sp., are poor survivors in the soil because of natural antibiosis and easy destruction by active saprophytic non-pathogenic soil bacteria (Gross and Vidaver, 1978). But several other reports implicate

survival of pathogenic bacteria in the soil along with plant debris for continuation of the disease in the next season. Such a relationship has been pointed out for bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Singh, 1971), and of red rot disease of witloof chichory caused by *Ralstonia syringae* pv. *marginalis* (Van-Outryve *et al.*, 1987).

Invariably the perpetuation pattern of the disease is also influenced greatly by continued cultivation of the same crop or any other intervening crop, which might be less susceptible. In another study Praşad and Sinha (1977a) reported that with cultivation of potato as a bridge crop between two maize crop the incidence of

stalk rot of maize due to *Erwinia chrysanthemi* pv. *zea* became very high.

A disease of rice in Ranchi caused by a bacterial pathogen was found, which had a distinctive disease syndrome quite different from the well known bacterial leaf blight of rice due to *Xanthomonas oryzae* pv. *oryzae*. We have reported the pathology of this disease of rice and have identified the causal organism as *Ralstonia marginalis* (Brown) Stevens (Kumar and Prasad, 1994; Kumar and Prasad; in Press).

It was, therefore, felt necessary to enquire into the ecology and survival pattern of the pathogen *R. marginalis*, infecting and causing marginal leaf spot of rice and barley.

MATERIALS AND METHODS

A study was designed for recording the survival pattern of the rice and barley pathogen *Ralstonia marginalis*. For this purpose, from the fields of rice and barley, soil samples were taken continuously at intervals of 25 days during the presence of the crop, and at intervals of 15 days after harvest. These samples were taken from 2.5 cm, 5 cm and 7.5 cm soil depth. The bacterial population in the soil was estimated after counting colonies on modified medium of Chaldecott and Preece (1983) without NaCl as an ingredient. Bacterial population from the leaf and stem portions of the crop standing in the field were also estimated at 25 days interval. The bacterial populations in the fallow field soil and the straw lying in the respective fields after harvest of rice and barley, were also estimated. The whole set of experiments were conducted for two years continuously from August till July next year. The soil samples were taken from the field where rice cultivars, IR-36, Rajendra 202 and Mussorie and barley cultivars, Karan 4, BAU-2205 and BR-32 were separately grown. Similarly survival on straw of each of the three rice and barley cvs. were estimated. The leaves and stems of rice and barley separately having lesions caused by *R. marginalis* were pooled in each instance and its bacterial population estimated. The bacterial numbers assessed in lesioned plant parts (stem and leaf), soil and straw were as per dilution plate method outlined earlier (Prasad and Sinha, 1977b).

RESULTS

Survival in soil and straw

As is evident from Table 1 maximum population of *R. marginalis* was invariably recorded from soil depth of 2.5 cm. This was true for the soil taken from fields

where each of the three rice and barley cvs. were separately grown. The population of the pathogen declined progressively with increase in soil depth.

The population of *R. marginalis* in the soil increased rapidly up to 75 days after transplantation of rice, being maximum in late September (Table 2 and Fig. 1). There was a sharp decline in its population after 100 and 125 days being much less by the end of the first week of November. In the rice straw and in the soil taken after 125 days of initial crop growth the bacterial populations were almost the same or slightly variable. In certain instances bacterial cells recovered were higher in the straw than in the soil. The population in the straw which could be slightly high or less in the soil was, however, sufficient for its survival.

During the intervening period between paddy harvest and sowing of barley, corresponding to November and early January (Table 2), overall decline in bacterial population took place. On the whole lower bacterial populations were apparent in the fallow field soils than those having standing crops. Although the population of *R. marginalis* was much less in the soil in the month of January than that recorded in late August to early November, still the amount of inoculum was sufficient enough to carry on the disease to the next cereal crop barley grown in the same field (Fig. 1). Comparatively high bacterial population was found in the soil with rice cv. IR-36 followed by those having Mussorie and Rajendra 202 (Table 2). The same trends were evident in the fallow field soil after harvest.

In the fields remaining fallow for six to seven weeks, three barley cvs. (Karan 4, BAU 2205 and BR-32) were sown in early January. In the soil samples taken at 25 days interval bacterial population increased progressively up to 75 days corresponding to end of January and the third week of March (Table 3 and Fig. 1). Thereafter the number of cells recovered in investigated soil samples diminished up to 125 days, corresponding to middle of April and early of May (Table 3, Fig. 1).

In the barley straw less bacterial population was apparent in the month of May than in the soil in the month of March (Table 3). In the fallow field soil after harvest of barley a progressive decline in bacterial population (at each 15 days interval) continued till the end of first week of July (Table 3). Still the number of bacterial cells was sufficiently high to perpetuate the disease in the next crop of rice. The inoculum also surviving in the left over barley straw, helped to carry over the disease on rice, which was sown in the same field (Fig. 1).

In the soil where barley cv. Karan 4 was grown

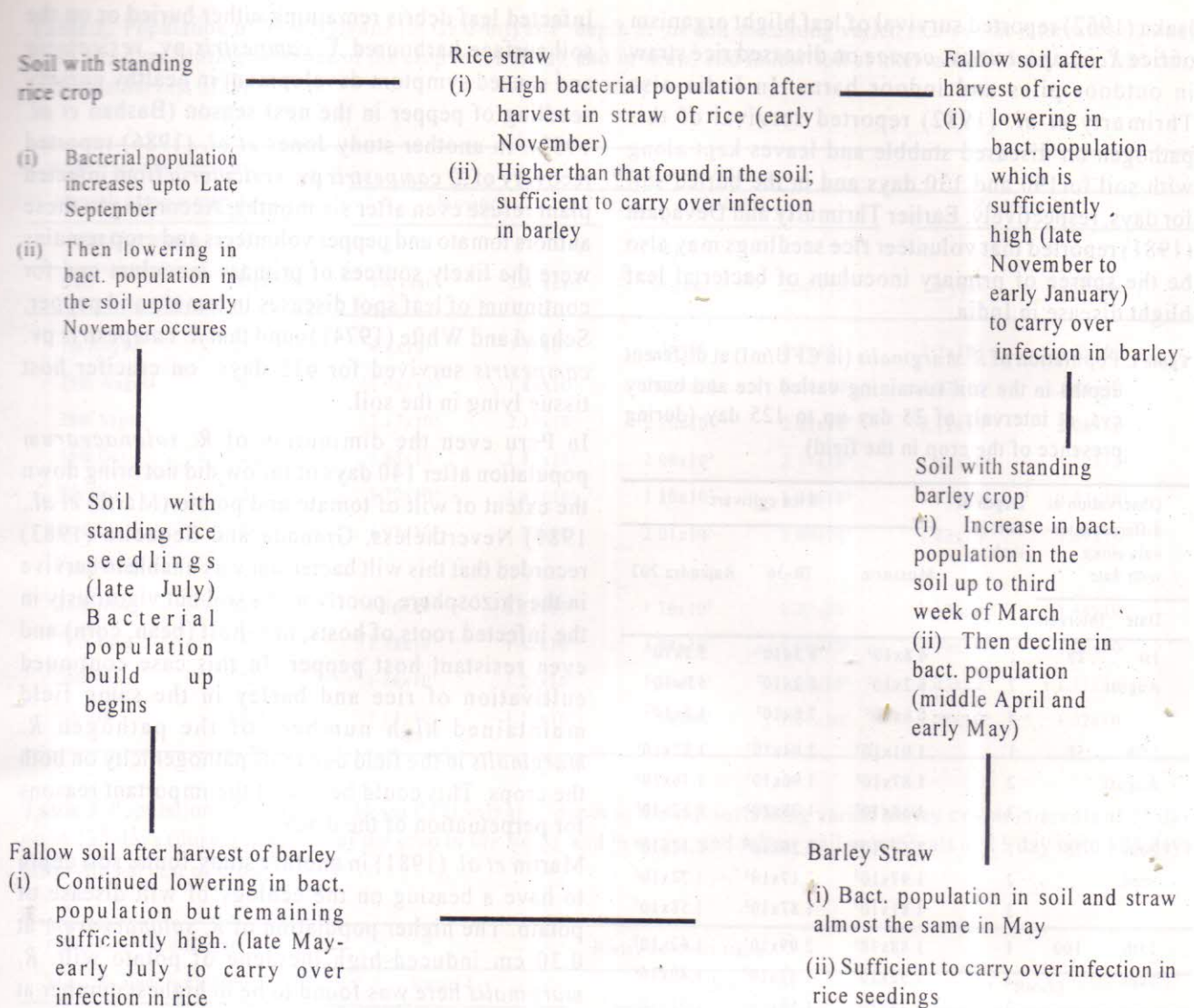


Fig. 1. Survival cycle of *Ralstonia marginalis* and its perpetuation in soil and straw of rice and barley.

maximum bacterial population was noted; less so in the soil having barley cv. BAU 2205 and the least in the soil with barley cv. BR-32 (Table 3). The same trends were discernible in the fallow field soil after harvest.

Similar survival pattern as found during August till next July was found in the fields of rice and barley grown next year.

Survival in stem and leaf lesions in the field during crop growth

As is evident from results presented in Tables 4 and 5 progressive increase in bacterial population took place in the stem and leaf lesion tissue of each of the rice and barley cultivars taken during 25-100 days of crop growth. Lesions present in rice cv. IR-36 (Table 4) and barley cv. Karan 4 (Table 5) had maximum bacterial population each. Comparatively the lesions

of leaves had higher bacterial population than those of the stem. This was true for all the cvs. of both rice and barley (Tables 4 and 5).

The inoculum buildup was parallel to maturation of the crop. Thus lesions with high bacterial populations might be the source of spread of the disease in the field.

Soil, straw, infected host-tissue and continued cropping of rice and barley in the same field, one after another, appear to be some of the important factors responsible for perpetuation of the disease from year to year (Fig. 1).

DISCUSSION

Plant debris, straw, stubble and plant refuses, all are abodes for surviving phytopathogenic bacteria and thus a role to play in perpetuation of diseases. In Japan,

Isaka (1962) reported survival of leaf blight organism of rice *X. campestris* pv. *oryzae* on diseased rice straw in outdoor piles and indoor barns. In India also Thrimurthy *et al.* (1982) reported survival of this pathogen on diseased stubble and leaves kept along with soil for 190 and 130 days and in the buried soil for days, respectively. Earlier Thrimurthy and Devadath (1981) reported that volunteer rice seedlings may also be the source of primary inoculum of bacterial leaf blight disease in India.

Table 1. Population of *R. marginalis* (in CFU/ml) at different depths in the soil sustaining varied rice and barley cvs. at intervals of 25 day up to 125 day (during presence of the crop in the field)

Observation at different intervals along with date	Depth of Soil in inch.	Rice cultivars			
		Mussorie	IR-36	Rajendra 202	
Date	Intervals				
1st August	25	1	6.8x10 ²	9.3x10 ²	5.2x10 ²
		2	6.2x10 ²	8.2x10 ²	5.0x10 ²
		3	6.6x10 ²	7.8x10 ²	4.8x10 ²
25th August	50	1	1.91x10 ³	2.04x10 ³	1.82x10 ³
		2	1.87x10 ³	1.96x10 ³	1.76x10 ³
		3	1.58x10 ³	1.78x10 ³	1.52x10 ³
20th Sept.	75	1	2.17x10 ³	2.26x10 ³	2.12x10 ³
		2	1.97x10 ³	2.17x10 ³	1.72x10 ³
		3	1.81x10 ³	1.87x10 ³	1.58x10 ³
15th Sept.	100	1	1.88x10 ³	2.09x10 ³	1.62x10 ³
		2	1.52x10 ³	1.67x10 ³	1.40x10 ³
		3	1.38x10 ³	1.58x10 ³	1.31x10 ³
9th Nov.	125	1	1.70x10 ³	1.80x10 ³	1.61x10 ³
		2	1.45x10 ³	1.57x10 ³	1.27x10 ³
		3	1.30x10 ³	1.32x10 ³	1.21x10 ³
		Barley cultivars	Karan 4	BAU-2205	BR 32
29th Jan.	25	1	1.05x10 ²	9.7x10 ²	7.06x10 ²
		2	8.9x10 ²	7.6x10 ²	6.5x10 ²
		3	7.9x10 ²	7.6x10 ²	6.1x10 ²
23th Feb.	50	1	1.83x10 ³	1.70x10 ³	1.61x10 ³
		2	1.75x10 ³	1.62x10 ³	1.54x10 ³
		3	1.57x10 ³	1.38x10 ³	1.30x10 ³
21st March	75	1	2.05x10 ³	1.94x10 ³	1.92x10 ³
		2	1.96x10 ³	1.76x10 ³	1.53x10 ³
		3	1.66x10 ³	1.60x10 ³	1.40x10 ³
15th April	100	1	1.88x10 ³	1.68x10 ³	1.42x10 ³
		2	1.46x10 ³	1.31x10 ³	1.19x10 ³
		3	1.38x10 ³	1.20x10 ³	1.12x10 ³
11th May	125	1	1.18x10 ³	1.60x10 ³	1.36x10 ³
		2	1.37x10 ³	1.25x10 ³	1.17x10 ³
		3	1.32x10 ³	1.17x10 ³	1.09x10 ³

Infected leaf debris remaining either buried or on the soil surface harboured *X. campestris* pv. *vesicatoria* and caused symptom development in healthy nursery seedling of pepper in the next season (Bashan *et al.* 1982). In another study Jones *et al.* (1986) reported recovery of *X. campestris* pv. *vesicatoria* from infected plant refuse even after six months. According to these authors tomato and pepper volunteers and crop remains were the likely sources of primary inoculum and for continuum of leaf spot diseases in tomato and pepper. Schaad and White (1974) found that *X. campestris* pv. *campestris* survived for 615 days on crucifer host tissue lying in the soil.

In Peru even the diminution of *R. solanacearum* population after 140 days of fallow did not bring down the extent of wilt of tomato and potato (Martin *et al.*, 1981) Nevertheless, Granada and Sequeira (1983) recorded that this wilt bacterium was unable to survive in the rhizosphere, poorly in the soil but vigorously in the infected roots of hosts, non-host (bean, corn) and even resistant host pepper. In this case continued cultivation of rice and barley in the same field maintained high numbers of the pathogen *R. marginalis* in the field due to its pathogenicity on both the crops. This could be one of the important reasons for perpetuation of the disease.

Martin *et al.* (1981) in another study found soil depth to have a bearing on the ecology of wilt disease of potato. The higher population of *R. solanacearum* at 0.30 cm induced high incidence of potato wilt. *R. marginalis* here was found to be in highest number at 2.5 cm soil depth; much more than the population at 5.0 cm and 7.5 cm soil depth. The high numbers near soil surface naturally were involved in carry over of marginal leaf spot disease in rice and barley.

Graham *et al.* (1979) found *R. solanacearum* race 3 in plant debris collected from heavily infected potato field even 33 weeks after the field was abandoned. The authors maintain that in cool temperate climate of New South Wales infected debris and latently infected self-sown tubers can be the sites for sustenance of the wilt pathogen. Kritzman and Zutra (1983) and Leben (1986) emphasized the role of plant debris, root and rhizosphere in survival of *R. solanacearum* pv. *lachrymans*. According to Kritzman and Zutra (1983) this bacterium survived in cucumber debris kept in wetted soil for 90 weeks. All the above reports conform to our findings regarding survival of *R. marginalis* in rice and barley straw.

In Kumaon hills of U.P. the summer grown potato had higher bacterial wilt incidence due to *R. solanacearum* than winter potato. Low temperature

Table 2. Population of *R. marginalis* (in CFU/ml) at 1" depth in the soil sustaining varied rice cvs. at intervals of 25 day up to 125 days (during presence of the crop in the field), and in straw, and fallow soil at intervals of 15 day upto 185 days (after the harvest of crop)

Observation at different intervals along with date		Rice cultivars taken					
		Mussorie		IR-36		Rajendra 202	
Under Rice crop							
Date	Intervals	1st year	2nd year	1st year	2nd year	1st year	2nd year
1st August	25	6.8x10 ²	7.1x10 ²	9.3x10 ²	8.7x10 ²	5.2x10 ²	6.8x10 ²
25th August	50	1.91x10 ³	1.88x10 ³	20.04x10 ³	1.94x10 ³	1.82x10 ³	1.81x10 ³
20th Sept.	75	2.17x10 ³	2.19x10 ³	2.26x10 ³	2.02x10 ³	2.12x10 ³	2.06x10 ³
15th Oct.	100	1.88x10 ³	1.87x10 ³	2.09x10 ³	2.17x10 ³	1.62x10 ³	1.72x10 ³
9th Nov.	125	1.70x10 ³	1.67x10 ³	1.80x10 ³	1.91x10 ³	1.61x10 ³	1.57x10 ³
Straw	125	1.95x10 ³	1.72x10 ³	2.01x10 ³	1.89x10 ³	1.82x10 ³	1.69x10 ³
Fallow soil							
25th Nov	140	1.56x10 ³	1.58x10 ³	1.76x10 ³	1.87x10 ³	1.30x10 ³	1.44x10 ³
10th DEc.	155	1.38x10 ³	1.42x10 ³	1.57x10 ³	1.66x10 ³	1.10x10 ³	1.22x10 ³
26th Dec.	170	1.26x10 ³	1.29x10 ³	1.39x10 ³	1.44x10 ³	1.03x10 ³	1.11x10 ³
9th Jan.	185	1.18x10 ³	1.19x10 ³	1.28x10 ³	1.37x10 ³	9.05x10 ²	1.02x10 ³

Table 3. Population of *R. marginalis* (in CFU/ml) at 1" depth in the soil sustaining varied barley cvs. at intervals of 25 day up to 125 days (during presence of the crop in the field), and in straw, and fallow soil at intervals of 15 day upto 185 days (after the harvest of crop)

Observation at different intervals along with date		Barley cultivars taken					
		Karan-4		BAU-2205		BR-32	
Under Barley crop							
Date	Intervals	1st year	2nd year	1st year	2nd year	1st year	2nd year
29th Jan.	25	1.05x10 ³	9.3x10 ²	9.7x10 ²	8.7x10 ²	7.6x10 ²	7.2x10 ²
23rd Feb.	50	1.83x10 ³	1.76x10 ³	1.70x10 ³	1.68x10 ³	1.61x10 ³	1.52x10 ³
21th March	75	2.05x10 ³	2.02x10 ³	1.94x10 ³	1.84x10 ³	1.92x10 ³	1.76x10 ³
15th April.	100	1.88x10 ³	1.94x10 ³	1.68x10 ³	1.73x10 ³	1.42x10 ³	1.47x10 ³
11th May	125	1.81x10 ³	1.82x10 ³	1.60x10 ³	1.68x10 ³	1.36x10 ³	1.47x10 ³
Straw	125	1.81x10 ³	1.62x10 ³	1.75x10 ³	1.58x10 ³	1.52x10 ³	1.48x10 ³
Fallow soil							
26th May	140	1.70x10 ³	1.67x10 ³	1.54x10 ³	1.62x10 ³	1.28x10 ³	1.43x10 ³
11th June	155	1.48x10 ³	1.41x10 ³	1.38x10 ³	1.45x10 ³	1.12x10 ³	1.37x10 ³
25th June	170	1.02x10 ³	1.24x10 ³	1.18x10 ³	1.21x10 ³	9.4x10 ²	1.16x10 ³
10th July	185	8.8x10 ²	1.05x10 ³	9.9x10 ²	9.3x10 ²	7.4x10 ²	9.3x10 ²

Table 4. Population of *R. marginalis* (in CFU/ml) obtained from stem and leaf lesion habitats of three rice cvs. at 25 day intervals up to 100 days and for two consecutive years

Observation at different intervals along with date	Year	Rice Cultivars					
		Stem			Leaf		
		Mussorie	IR-36	Rajendra	Mussorie	IR-36	Rajendra
		202			202		
25	I	1.20 x 10 ³	1.40 x 10 ³	1.02 x 10 ³	1.43 x 10 ³	1.50 x 10 ³	1.29 x 10 ³
1st August	II	1.31 x 10 ³	1.33 x 10 ³	1.16 x 10 ³	1.48 x 10 ³	1.61 x 10 ³	1.32 x 10 ³
50	I	1.31 x 10 ³	1.52 x 10 ³	1.16 x 10 ³	1.60 x 10 ³	1.62 x 10 ³	1.49 x 10 ³
1st August	II	1.35 x 10 ³	1.48 x 10 ³	1.27 x 10 ³	1.58 x 10 ³	1.69 x 10 ³	1.52 x 10 ³
75	I	1.43 x 10 ³	1.67 x 10 ³	1.28 x 10 ³	1.74 x 10 ³	1.80 x 10 ³	1.61 x 10 ³
20th Sept.	II	1.41 x 10 ³	1.57 x 10 ³	1.32 x 10 ³	1.67 x 10 ³	1.82 x 10 ³	1.64 x 10 ³
100	I	1.57 x 10 ³	1.81 x 10 ³	1.42 x 10 ³	1.89 x 10 ³	1.97 x 10 ³	1.76 x 10 ³
15th Oct.	II	1.52 x 10 ³	1.69 x 10 ³	1.39 x 10 ³	1.82 x 10 ³	1.93 x 10 ³	1.77 x 10 ³

Table 5. Population of *R. marginalis* (in CFU/ml) obtained from stem and leaf lesion habitats of three barley cvs. at 25 day intervals upto 100 days and for two consecutive years

Observation at different intervals along with date	Year	Barley Cultivars					
		Stem			Leaf		
		Karan 4	BAU-2205	BR-32	Karan 4	BAU-2205	BR-32
25	I	1.22x10 ³	1.12x10 ³	9.1x10 ³	1.39x10 ³	1.29x10 ³	1.04x10 ³
29th Jan.	II	1.24x10 ³	1.19x10 ³	9.7x10 ³	1.28x10 ³	1.22x10 ³	1.15x10 ³
50	I	1.39x10 ³	1.27x10 ³	1.04x10 ³	1.56x10 ³	1.46x10 ³	1.15x10 ³
23rd Feb	II	1.32x10 ³	1.09x10 ³	1.04x10 ³	1.47x10 ³	1.36x10 ³	1.29x10 ³
75	I	1.53x10 ³	1.39x10 ³	1.17x10 ³	1.72x10 ³	1.60x10 ³	1.27x10 ³
21st March	II	1.48x10 ³	1.37x10 ³	1.25x10 ³	1.63x10 ³	1.49x10 ³	1.34x10 ³
100	I	1.68x10 ³	1.56x10 ³	1.30x10 ³	1.86x10 ³	1.72x10 ³	1.42x10 ³
15th April	II	1.62x10 ³	1.48x10 ³	1.39x10 ³	1.72x10 ³	1.66x10 ³	1.56x10 ³

and low humidity reduced bacterial multiplications. However high humidity and high temperature favoured bacterial multiplication and its infectivity with enhanced disease incidence (Sunaina and Gupta, 1998). Here in rice and barley no such co-relationship between high temperature and high humidity and high incidence of disease due to *R. marginalis* could be seen.

The bacterial pathogen, *R. marginalis*, however, survived for a limited duration in the fallow soil and straw only to bridge over the period when rice and barley crops have been harvested.

It could be thus summarized that the pathogen *Ralstonia marginalis* causing marginal leaf spot disease of rice and barley, occur, survive and proliferate in large numbers in leaf and stem tissue lesions of three cvs. each of rice and barley. The prominently high populations of *R. marginalis* in aereal lesions of leaf and stem are responsible for spread of the disease in the field.

For perpetuation of the disease next year *R. marginalis* continuously proliferated in the soil with the standing crops rice and barley grown one after another and then survived in their straw, and the fallow soil after their harvest. This could be the reason for maintenance of the disease cycle year after year.

ACKNOWLEDGEMENT

We thank Professor K.K. Nag, Head, University Department of Botany, Ranchi University, for laboratory facilities.

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(Accepted for publication February 27, 2001)

INTRODUCTION

The bacterial wilt disease of maize is caused by the bacterium *Erwinia carotovora* f. sp. *zeae* (Prasad and Sinha, 1977). The disease is characterized by wilting and death of the plant. The bacterium is soil-borne and enters the plant through the roots. The disease is common in maize grown in the tropics and subtropics. The bacterium is also reported from maize in India (Prasad and Sinha, 1977). The disease is caused by the bacterium *Erwinia carotovora* f. sp. *zeae* (Prasad and Sinha, 1977). The bacterium is soil-borne and enters the plant through the roots. The disease is common in maize grown in the tropics and subtropics. The bacterium is also reported from maize in India (Prasad and Sinha, 1977).

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