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Biotype Distribution of Vascular Wilt Pathogen *Pseudomonas solanacearum* in Sri Lanka

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Abstract: Pathotypes of *Pseudomonas solanacearum*, Smith were collected from various agroecological regions of Sri Lanka. Only two biotypes sensu Hayward, namely biotype 2 and 3 were found.

Biotype 2 was isolated from potato plants grown in the central highland and it was found to be delimited to isotherm 16°C. Biotype 3 which was pathogenic to all solanaceous crops, was isolated from almost all the sampling sites throughout the island. The wet and dry zone climatic regions did not, apparently have any effect on the distribution of biotypes.

Many samples of *P. solanacearum* in the hill country dry zone were found to carry *P. marginalis*, which had a synergistic effect on the production of wilt disease. *P. marginalis* showed similar biochemical reactions as biotype 4 of *P. solanacearum*.

1. Introduction

The bacterial wilt caused by *Pseudomonas solanacearum* E. F. Smith is one of the major diseases of solanaceous crops. The disease occurs mostly in warm climates. It is known to infect various other hosts such as Banana[11] Peanuts[9], Caster[12], Winged bean[1] and has an erratic distribution in soil.[8] Also variation within the species based on morphological, physiological properties has been well documented.[2,4,7,13,15]

Hayward[5] grouped *P. solanacearum* into four biotypes. This classification was based on their ability in oxydizing disaccharides-lactose, maltose, cellobiose and hexose alcohols - mannitol, sorbitol and dulcitol. Seneviratne[10] reported an occurrence of biotypes 2, 3, and 4 in the hill country of Sri Lanka. Further studies were done to establish the distribution of biotypes throughout major agricultural areas of the island. An attempt was made to relate it to environmental factors.

2. Materials and Methods

2.1 Field survey

Solanaceous crops infected with *Pseudomonas solanacearum* Smith were collected from various agroecological regions of Sri Lanka. Whole plant samples were brought to the laboratory and vascular tissues were used for the isolation of pathogen from infected tomato, potato, capsicum and brinjal plants. Potato tubers were also used for the isolation of bacterium.

Selection of sampling locations were based on the previous cropping history, soil type, altitude, rainfall and temperature of the region.

The popular climatic division of the island is classified into two major divisions "wet" and "dry" zone. This expresses the regional hygro-climatic differentiation based on climatic reality which also reflects a land use and crop cultivation pattern. Although the wet and dry zones (Figure 1) are not internationally valid terms defined by climatic indices, they are realistic to climatic situation in the island. Of course viewed from the other dry regions of the earth the "Driest" in the dry zone - Mahalewaya - saltern in Hambantota still records an average rainfall of 929 mm per year.

In the present study agro-ecological regions classified according to 75% expectancy values of annual rainfall and elevation (Land and Water in 1979) was used for the selection of sampling locations. This apparently coincides with the wet and dry zone classification proposed by Wickramatilaka (1963) on effective dry period basis (Figure 1).

The land area under wet and dry zones are 1.6 million and 4.9 million ha respectively. The average annual rainfall in wet zone is about 32 million acre feet (1 Ac.ft. = 760 mm/ha) and 57 million acre feet in the entire island, that is rainfall in the wet zone is 72% higher over dry zone.[3]

2.2 Dry Zone

In the dry zone, wet and dry periods alternate once a year. The rainy period is only 3 - 4 months (Oct./Nov. to January) but the rains are disproportionately high. The dry period, maximum of 8 months (Feb. to Sept./Oct.) is extremely dry. The 75% expectancy value of annual rainfall is around 508 - 762 mm.

Soils in the dry zone are mainly reddish brown earths. But red yellow latosols and regosols, low humic gley soils and solodized solonitz are also found in some regions (DL_4).

The annual average temperature however does not relate to dry and wet conditions. The average annual records reveals homogenous temperature in the lowlands and the rapidly decreasing temperature in the highlands. In the lowland dry zone up to 150 m the temperature varies from 26.5^{0}to 28^{0}C, the spatial variations of temperature in the region are slight.

These climatic conditions have produced a suitable niche to many crops and a wide variety of vegetation in the dry zone. Many grain crops, pulses, vegetables of various families, tubers and industrial crops are the major economic crops grown in the area. Among the common annual crops, rice is by far the most important crop during Maha. Although the soils are flooded for 3 - 4 months during paddy crops, solanaceous vegetables mainly capsicum, tomato and brinjal are grown under irrigation in these fields during the Yala season. However, in Jaffna potato crop is given high priority.

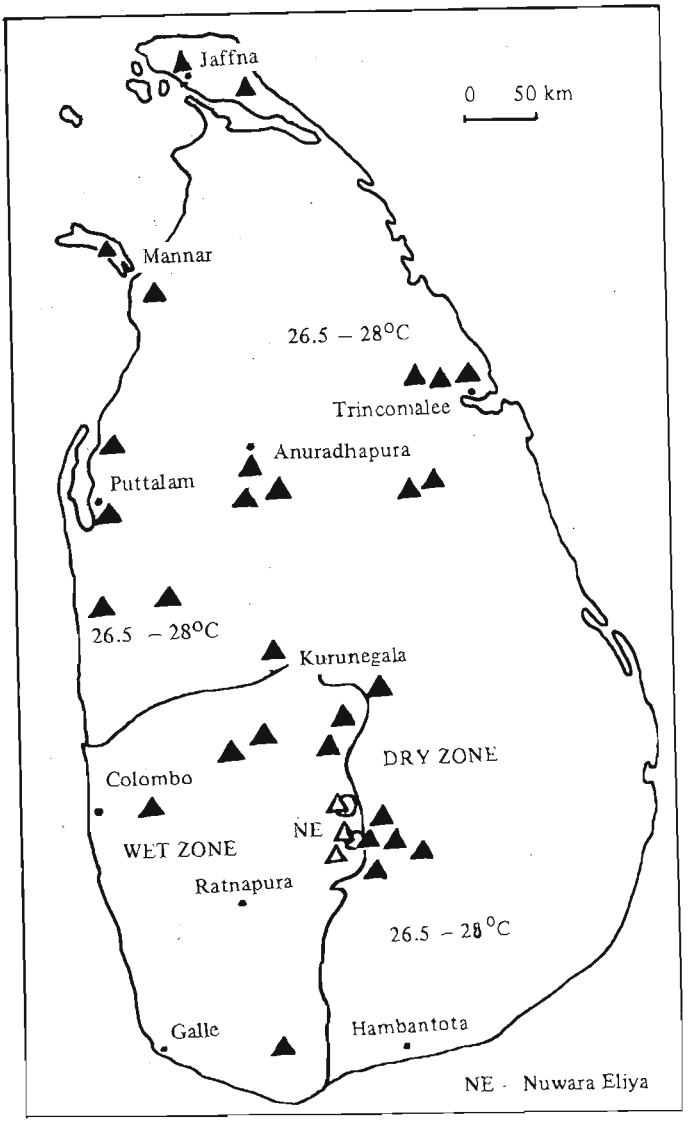

Fig. 1. The distribution of Pseudomonas solanacearum biotypes in Sri Lanka.

▲ – Biotype 3 (1979–1982)
△ – Biotype 2 (1979–1982)
O – Biotype 2 (Seneviratne 1969)

Samples of naturally infected plants of potato (*Solanum tuberosum* L) and tubers from Jaffna - Thirunelvely, 1981 and Trincomalee - Kuchchavely and Uppuvely 1980, brinjal plants (*Solanum melongenae* L) from Jaffna - Kondavil, 1979, Trincomalee - Pankulam, 1980, Mannar - 1979, Vanathavillu - 1981, Puttalam - 1981, Monaratenne, Divlana - 1981, Bingiriya - 1981, capsicum (*Capsicum annum* var. *grossum*(L)) from Kandalama - 1980, Madatugama 1980, Mahailluppallama 1980, Kalawewa - 1982 were collected. Five isolations were made from at least 4 specimens from each farm.

2.3 Wet Zone

The Wet zone - Dry zone boundary that runs from Negombo via Kurunegala, and then to Matale, along the top ridge of Knuckles through the Corbet's gap to Pidurutalagala massif to go south of Nuwara Eliya across the high plains. Then it goes round Balangoda in an arc along the Sabaragamuwa hill country to reach Matara. The South West quarter of the island thus forms the Wet zone. However, the boundary is not rigid and merge with dry zone through the intermediate zone.

The 75% expectancy values of annual rainfall range from 889-3175 mm. Soils are mainly red yellow podzolic type. Variation on the horizons are found.

Table 1.– Annual average (1931-1960) temperature at various observation stations, closer to the sampling locations selected.

Observation stations closer to sampling site					
	DRY ZONE			WET ZONE	
Station	Elevation	Mean C	Station	Elevation	Mean C
	m			m	
Puttalam	2	27.2	Colombo	7	26.9
Batticaloa	3	27.4	Galle	13	26.5
Mannar	4	27.8	Kandy	477	24.4
Jaffna	4	27.6	Talawakele	1375	18.6
Anuradhapura	93	27.2	Nuwara Eliya	1882	15.4
Kurunegala	116	27.0			
Hakgala	171	17.3			

From a climatic standpoint, the wet zone is thus characterized by greater cultivation advantages in terms of more intensive diversified land use. In the Pidurutalagala massif, the central highlands of Sri Lanka rise to a maximum altitude

of 2,524 m, which produces a considerable vertical thermal contrast between highlands and lowlands. Temperature variations are very prominent, it falls quickly as the altitude increases (Table 1).

Because of the thermal requirements, the optimum condition for wetland rice occurs in the hill country only up to about 1,200 m (4,500 ft). However, other crops such as potato, tomato, brinjal and capsicum are grown. New areas have been cleared for potato cultivation in the hill country.

Samples were collected from potato fields either grown in traditional potato fields, newly opened areas or paddy fields. Potato tubers and plants were collected from Hawaeliya – 1981. Nuwara Eliya – 1981, Sita Eliya – 1981, Rahangala – 1981 and Yalapatwela – 1981. Infected tomato plants were obtained from Kandy, University farm – 1980. Diseased tomato plants were also collected from Rahangala – 1981, and Bandarawela – 1981. Bacterium was also isolated from the tomato samples collected from Mirahawatte and Welimada – 1981.

Several samples from each location were used for the isolation of the pathogen. The tomato samples collected from low country wet zone Weboda (1979), Mawanella 1979 , and Mapalana 1980 were also used for this study.

2.4. Isolation of the Pathogen

Isolations were made from potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill), Egg plant (*Solanum melongenae* L) and capsicum (*Capsicum annum* var. *grossum*) plants showing typical symptoms of bacterial wilt in the field. Bacterium was isolated from the vascular tissues of infected stem and was used to streak plants. Potato tubers from infected plants were also used for the isolation of the pathogen. Bacterial ooze from clean freshly cut surface of tubers were taken in sterile water as inoculum.

Axenic cultures were prepared by streaking agar plates with the bacterial samples. The medium used for culturing and routine maintenance was as follows: (calcium carbonate agar) peptone 5.0 g; yeast extract 0.5 g; glucose 5.0 g; K_2HPO_4 0.2 g; $MgSO_4.7H_2O$, 0.2g; $CaCO_3$, 1.0 g; agar 18.0 g; distilled water 1 L. Nutrient medium was sterilized at 15 psi for 20 min.

Inoculated plates were maintained at 21 ± 0.1°C in precision low temperature incubators.

2.5 Pathogenicity tests

Pathogenicity of bacterial isolations were confirmed by reinoculating tomato plants. Five weeks old tomato cv. Marglobe plants raised in sterile soil in plastic pots (15.5 cm diameter) were stem inoculated with bacterial suspension containing 10^6 cells/ml. Bacterial suspension was prepared with 48 h cultures grown in calcium carbonate agar. Three 10 μ l drops of the suspension was placed on the axil of the third leaf from

the top, and the stem was then pricked with a syringe needle (gauge 25) through the inoculum drop.

Inoculated plants were placed in the green house (24^{0}C night, 28^0- 33^{0}C day). Plants were observed for the wilt and yellowing after 3 days from inoculation.

2.6 Biotype separation

All isolations were subjected to biochemical tests for their ability to oxidize disaccharides and hexose alcohol proposed by Hayward[5] Bacterial cultures were inoculated to culture tubes (1 x 12.5 cm) containing the following medium.

Basal medium per litre: $NH_4H_2PO_4$, 1.0 g; KCL 0.2 g; $MgSO_4.7H_2O$ 0.2 g; yeast extract 1.0 g and Bromothymol blue 0.3 ml of 1% W/V solution in 50% ethanol. Medium was adjusted to pH 7.1 with 1N NaOH before adding 1.5 g agar.

To 9 parts of sterile, cooled basal medium added 1 part of membrane filter sterilized 10% (w/v) of each carbon source.[5] Medium was then dispensed aseptically in a horizontal Laminar Floor Cabinet (Environmental Control INC.) to sterile plugged tubes to a depth of about 4 mm.

Inoculated slopes were incubated at 27^{0}C for 14 days. Slopes were observed for acid production on the 3rd, 7th and 14th day before discarding.

3. Results and Discussion

It was found that in the Yala season (N. E. monsoon, December - February) *Solanum melongenae* L. (egg plant) was the dominant solanaceous crop grown in the Dry Zone farms visited. *Pseudomonas solanacearum* isolated from diseased plant from almost all the sites except Kuchchavely in Trincomalee were pathogenic strains. The wilting of potato samples collected at Kuchchavely was due to *Fusarium oxysporum* and other *Fusarium spp.*

In the biochemical tests, the development of a yellow colour in the medium indicated production of acid from the oxidation of carbon sources. In most cases where there is positive acid production slight yellow colour appeared around inoculum by the 3rd day and it was yellow throughout the medium after 1 week of growth at 27^{0}C.

The distribution of biotypes in various agroecological regions is summarized in Figure 1. Heyward grouped *P. solanacearum* into 4 biotypes. Biotypes 3 and 2 were recorded in both dry and wet zone of Sri Lanka. However, biotype 2 was restricted to hill country.

Potato samples mainly tubers collected at Uva basin 1200 - 1400 M, rice fields (Rahangala - 80%, Boralanda - 30%, Yalapatwala - 85% and Mirahawatte - 30%) and samples from Sita Eliya - 20% and Hawaeliya - 15%, showed positive response to biotype 4 in biochemical tests.

Although this confirms to the results previously reported by Seneviratne[10] from the samples collected at Gorandiyatenna, it was decided to subject the bacterium for further biochemical and taxonomical study.

The biochemical tests used were Oxidase test with a platinum loop[6], Arginine dihydrolase test[14] Nitrate reduction and Carbon source utilization in Ayers *et al.* mineral salts medium in addition to Acid production tests.[5] Results are summarized in table 2.

Table 2.–Differentiation of *Pseudomonas solanacearum* isolates from Uva basin, Sita Eliya and Hawaeliya.

Test	*Pseudomonas solanacearum* isolates						
	Biotype 2	Biotype 3	Standard Biotype 4	R	B	Y	HE
Arginine hydrolase	ND	ND	ND	V^+	+	+	V^+
Oxidase test	+	+	+	V^+	+	+	+
Nitrate reduction	-	-	-	V^+	+	+	V^+
Carbon source for growth							
Cellobiose	-	-	-	-	-	-	-
Trehalose	-	+	+	+	+	+	+
Mannitol	-	+	+	+	+	+	+
Sorbitol	-	+	+	+	+	+	+
Acid production tests							
Lactose	A	A	-	V	V	V^+	V^+
Maltose	A	A	-	V	V	V^+	V^+
Mannitol	-	A	A	A	A	A	A
Sucrose	A	A	A	V	V^+	V^+	V
Sorbitol	-	A	A	A	A	A	A

ND—Not detected
V —Variable
R —Rahangala
B —Boralanda
Y —Yalapatwela
HE—Hawaeliya

\+ Definite positive response
V^+ Slight positive response
\- No response or no acid production
A Acid producing.

Although the carbon source utilization and acid production tests indicated a presence of biotype 4, it is evident from table 2 that the results are not conclusive enough. Therefore all isolates were then grown at 27^{0}C in King's medium B agar (oxidase positive), composed of proteose peptone (Difco) 20.0 g; $K_2HPO_4.3H_2O$ 2.5 g; $MgSO_4.7H_2O$ 6.0 g; agar 15.0 g; glycerol 15.0 ml in 1 L of distilled water and examined with a long wave (375 nm) ultra violet lamp Gelman, universal UV unit for fluorescence. Green fluorescence was detected.

Fluorescent and the non-fluorescent were later separated out carefully for pathogenicity tests. Non-fluorescent bacteria was then identified as pathogenic *Pseudomonas solanacearum* biotype 3 whereas fluorescent bacteria was identified as *Pseudomonas marginalis*. When artificially inoculated tubers showed soft rot, and slight wilting of plant with browning of leaf margins. However when both strains were inoculated to potato plants wilting was severe and prominent, with conspicuous vascular wilt symptoms.

It is evident from these results that in the hill country wet and intermediate zone (IV_2 & IV_3) *Pseudomonas marginalis* prevails in association with *Pseudomonas solanacearum*. Probably it has a synergistic effect in wilt production.

Although Seneviratne (1964) reported the presence of biotype 4 in the hill country, I have found only 2 biotypes namely 2 and 3 among the samples collected. The distribution of biotype 2 was restricted to potato and within the 16^{0}C isotherm (Figure 2). Absence of biotype 2 in potato crops in the low land dry zone holds evidence for the effect of temperature on the survival. Figure 3 represents the thermal diurnal climate of two representative stations Colombo - Low country and Nuwara Eliya for hill country. Thermo isopleth diagrams of Colombo and Nuwara Eliya show the corresponding course of the isopleths. However, the order of temperature magnitudes are different. The maximum and minimum temperatures are shown during the period from December to May. Lowest temperatures are early in the mornings and highest at 12 - 14 hrs. However, onset of rains has minor effect on the diurnal course. The temperature remains constant from 12 - 17 hrs in the hill country Nuwara Eliya region it is about 18 - 23 ^{0}C and in Colombo 29 - 31.3^{0}C in December - January. However in Nuwara Eliya range is 11 - 17^{0}C during the rest of the year whereas it remains high around 22 - 29.2^{0}C.

During the main potato cropping season in the hill country October/November to February and February/March to July has relatively low temperature periods compared to 11 - 23^{0}C during February/March period. It is possible that 11 - 17^{0}C is favourable for epiphytic development of the bacteria in the field. Although the temperature could go up to about 23^{0}C during February/March it is only for a period of 2 hours (12.00 to 14.00 hours). Therefore it is possible that the population of biotype 2 restricted to potato crop could survive in the areas without much losses during land preparation and planting in February - March.

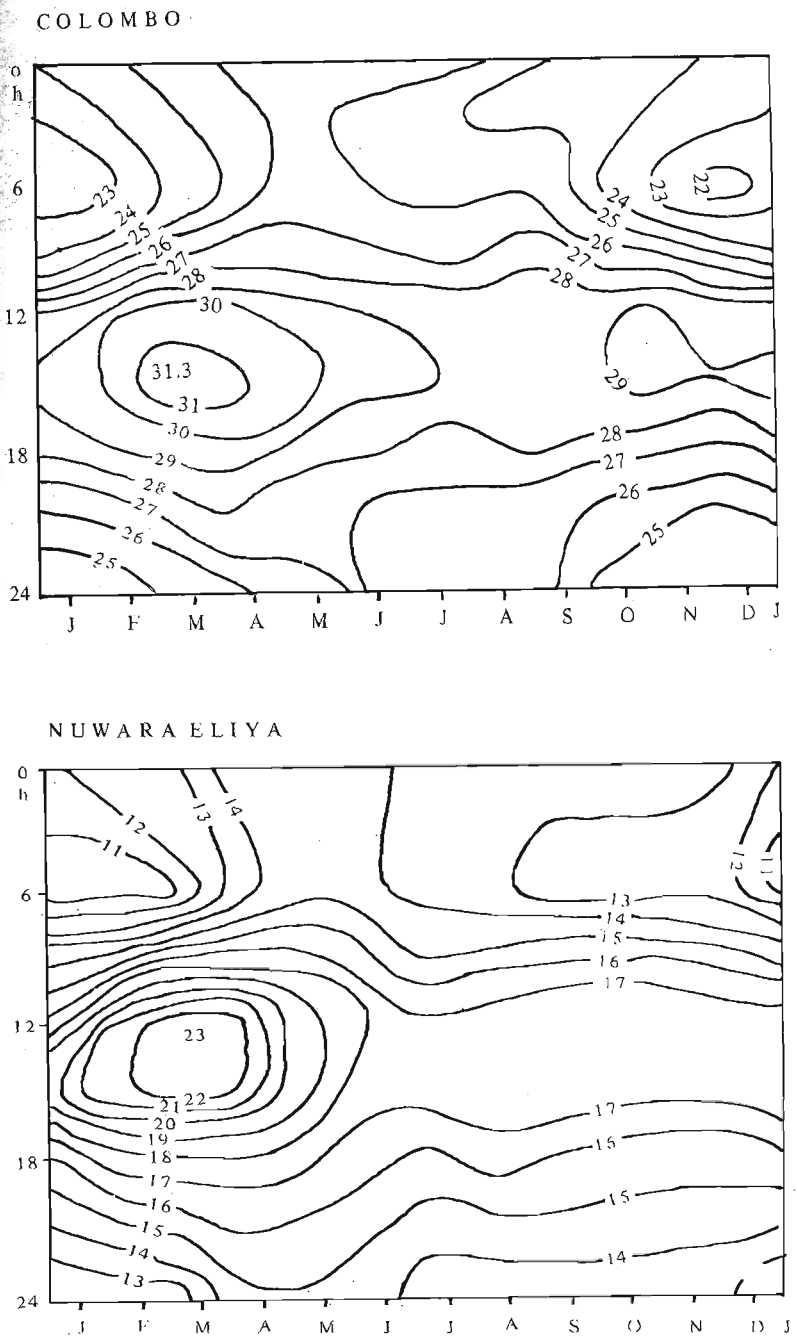

Figure 2. Thermo-isopleths diagrams for Colombo and Nuwara Eliya.

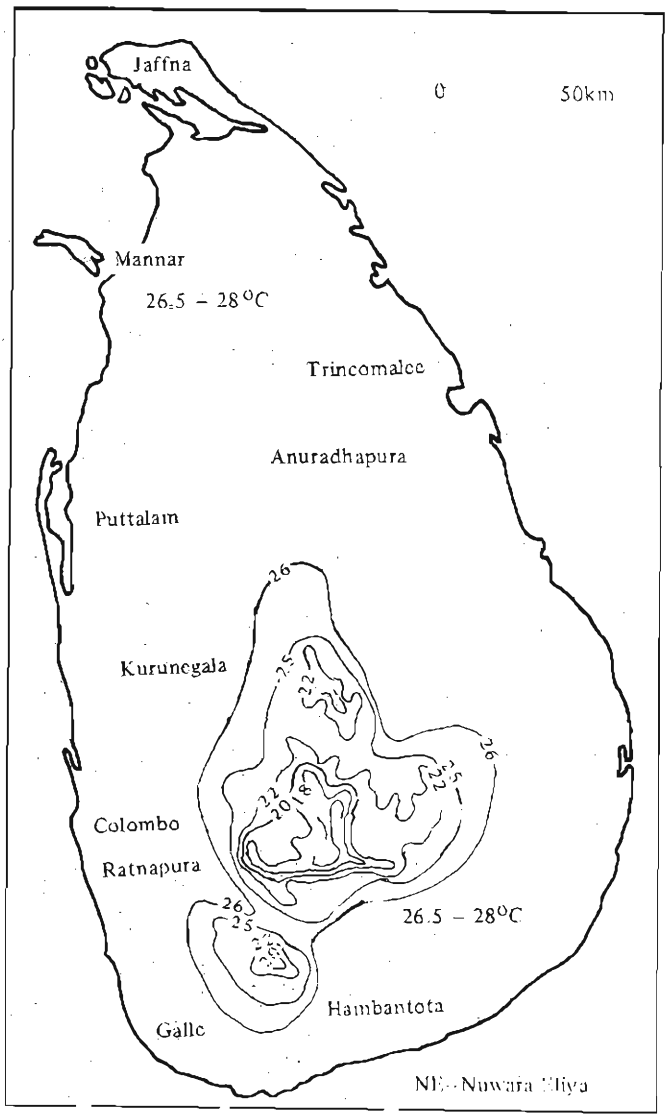

Figure 3 – Map of the annual average temperatures in Sri Lanka, isotherms in °C.

Seneviratne[10] suggested a correlation between cropping history, soil type and biotype occurrence. The samples collected from the farms in new clearings of the Kalawewa and Madatugama (Capsicum) showed biotype 3 only. Only biotype 3 was isolated from intermediate zone $1V_3$, $1M_3$ and $1L_1$ agroecological regions. Even in the new clearings and in potato crops in paddy fields at Yalapatwela only biotype 3 was detected.

The characteristic feature of the distribution of biotype 2 is that it is restricted to a small area in the hill country delimited by 16^0C isotherm. This indicated its temperature dependency rather than rainfall or soil types.

In view of the fact that both biotypes 2 and 3 were found in new clearings and its occurrence in the hill country where the distribution through irrigation water is unlikely it is suggested that these biotypes are indigenous to Sri Lanka. The broad spectrum host range of biotype 3 and its ability to survive even at higher temperatures such as 31^0C has made the occurrence of biotype 3 islandwide.

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