

Studies on phyllosphere Fungi.

VII. Foliar application of urea on certain ornamentals

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Leaf-surface mycoflora of three ornamentals — *Impatiens balsamina* L., *Rhees discolor* L. and *Lochnera rosea* (L.) Reichb., in relation to foliar application of aqueous solution of 5,0% urea has been studied. In the former two plants the application of urea enhanced the mycoflora whereas in the latter an adverse effect was observed for phyllosphere region. In phylloplane region of *L. rosea*, however, a promotive effect was exhibited in sprayed plants. The urea in this concentration also proved slightly toxic to *L. rosea*.

INTRODUCTION

The work of Last (1955), Ruinen (1961) and Dickinson (1965) resulted in a number of investigations (Dickinson 1965, 1967; Sinha 1965; Sol 1966, 1967, 1968; Mishra, Kanaujia 1971, 1974) on leaf surface microbiology. Most of the previous workers stated above have reported the phyllosphere mycoflora in relation to plant age, viral and fungal infection and antagonisms. Very little is known about the effect of foliar sprayed substances on leaf surface mycoflora (Kanaujia 1975a, b; Mishra, Kanaujia 1975; Sol 1966, 1967). Sol (1966, 1967, 1968) has reported the pretreatment of leaves of *Vicia faba* with certain inorganic and organic substances like sucrose, potassium chloride, lathanum chloride and decenyl succinic acid has increased the lesions of *Botrytis fabae* per unit area of leaf. Studies, however, regarding the phyllosphere and phylloplane mycoflora in relation to foliar application on substances like urea have not yet been perfounded and this has been tried in the present investigation.

Table 1

Distribution of fungal species in the phyllosphere/phyllplane regions of urea sprayed plants (A) and control (water sprayed — B, unsprayed — C) plants of certain ornamentals and adjacent air

Fungi species	Ornamentals												Atmo- spheris air				
	<i>Impatiens balsamina</i>			<i>Rhoeo discolor</i>			<i>Lochnera rosea</i>			C							
	A	B	C	A	B	C	A	B	A								
<i>Rhizopus stolonifer</i> (Ehr.) Vuill. (= <i>R. nigricans</i> Ehr.)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Mucor hiemalis</i> Wehm.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Mucor</i> sp.																	
<i>Cunninghamia elegans</i> Lendner (= <i>C. bertholletiae</i> Stadel)	+		+														
<i>C. echinulata</i> (Thaxter) Thaxter																	
<i>Chaetomium globosum</i>																	
<i>C. herbarum</i>																	
<i>Trichoderma viride</i> Pers.																	
<i>Monilia sitophila</i> (Mont.) Sacc.																	
<i>Aspergillus aculeatus</i> Iizuka																	
<i>A. flavipes</i> (Bain. and Sart.) Thom and Church																	
<i>A. fischeri</i> Wehmer																	
<i>A. flavus</i> Link																	
<i>A. fumigatus</i> Fresenius,																	
<i>A. niger</i> Van Tieghem																	
<i>A. phoenicis</i> (Cda.) Thom																	
<i>A. terreus</i> Thom																	
<i>A. versicolor</i> (Vuill.) Tiraboschi																	
<i>P. chrysogenum</i> Thom																	

MATERIALS AND METHODS

Impatiens balsamina L. (IB), *Rheoes discolor* L. (RD) and *Lochnera rosea* (L) Reichb. (LR) extensively cultivated ornamentals were selected in the present study. Thirty days old seedlings of equal size were separately transplanted in the kitchen garden of the author. The first spraying of 5.0% aqueous solution (w/v) was carried out when plants were 45 days old. Twenty ml solution was sprayed per plant separately 15 days apart and sampling of leaves from the 5 replicats kept for one ornamental was done 24 h after spraying. The soil was covered by paper to avoid the contact of the solution with it. Simultaneously, control plants were sprayed with an equal volume of sterile distilled water (A). Another control (B) was kept where plants were left unsprayed. The spraying was done on foliage in the evening. The experiment was harvested in 121 days. The collection of leaves and isolation of phyllosphere and phylloplane mycoflora was carried out as described by Mishra and Srivastava (1971). The fungi of adjacent atmospheric air were also recorded. The population was expressed on the basis of per sq. cm. area of leaf surface and per cm³ of nutrient plates in phyllosphere and air respectively. The findings are presenter in Tables 1-3.

RESULTS

Thirty-four fungal species comprising 5 phycomycetes, 2 ascomycetes 22 deuteromycetes and 5 sterile fungi were isolated from phyllosphere, phylloplane regions of three ornamentals both sprayed and controls and adjacent atmospheric air (Table 1). *Mucor hiemalis*, *Trichoderma viride*, and *Aspergillus flavus* were obtained in the phyllosphere and controls of all the three plants. Only *Aspergillus flavus* was commonly present in the phylloplane of all the plants. Other frequently isolated fungi were *Aspergillus aculeatus*, *A. fumigatus*, *A. terreus* and *Alternaria tenuis*. Few isolates were specifically distributed to different ornamentals (Table 1).

In urea sprayed phyllosphere region, the number of fungal species was highest in *I. balsamina* and almost high in *R. discolor*. Qualitatively fungi were least in treated plants of *L. rosea*. Urea sprayed phyllosphere and phylloplane region IB and RD exhibited a higher number of fungi than their respective controls. The pattern was reversed for LR in phyllosphere whereas the number of fungi in phylloplane region of this plant was appreciably increased by urea spraying. Two controls kept for each ornamental showed marginal variation (Table 1).

Table 2 revealed that fungal species on different sampling dates be-

Table 2

Distribution of species in phyllosphere/phyloplane regions of urea sprayed (A) and control plants (water sprayed—B, unsprayed—C) plants of certain ornamentals and adjacent air

Sampling dates	Ornamentals									Adja- cent air
	<i>I. balsamina</i>			<i>R. discolor</i>			<i>L. rosea</i>			
	A	B	C	A	B	C	A	B	C	
July 1, 1975	$\frac{8}{3}$	$\frac{6}{3}$	$\frac{6}{3}$	$\frac{7}{1}$	$\frac{3}{-}$	$\frac{3}{-}$	$\frac{6}{2}$	$\frac{5}{2}$	$\frac{4}{2}$	25
July 16, 1975	$\frac{14}{5}$	$\frac{8}{3}$	$\frac{7}{3}$	$\frac{9}{2}$	$\frac{4}{1}$	$\frac{5}{1}$	$\frac{5}{3}$	$\frac{6}{2}$	$\frac{6}{1}$	23
August 15, 1975	$\frac{16}{7}$	$\frac{8}{4}$	$\frac{7}{3}$	$\frac{10}{3}$	$\frac{6}{2}$	$\frac{5}{2}$	$\frac{4}{5}$	$\frac{8}{2}$	$\frac{7}{2}$	20
July 31, 1975	$\frac{17}{8}$	$\frac{9}{4}$	$\frac{9}{4}$	$\frac{15}{4}$	$\frac{7}{3}$	$\frac{6}{2}$	$\frac{4}{6}$	$\frac{9}{3}$	$\frac{10}{3}$	23
August 30, 1975	$\frac{18}{8}$	$\frac{10}{4}$	$\frac{9}{4}$	$\frac{17}{5}$	$\frac{7}{3}$	$\frac{7}{3}$	$\frac{3}{7}$	$\frac{11}{3}$	$\frac{10}{4}$	22

Table 3

Fungal population in phyllosphere (per sq. cm area of leaf) and phyloplane (No. of cols/plate) of urea sprayed (A) and control (water sprayed—B and unsprayed—C) plants ornamentals and in adjacent atmospheric air (cols/per cm² area of plate surface)

Sampling dates	Ornamentals									Adja- cent atmo- spher- e (cm ²)
	<i>I. balsamina</i>			<i>R. discolor</i>			<i>L. rosea</i>			
	A	B	C	A	B	C	A	B	C	
July 1, 1975	$\frac{185}{4}$	$\frac{150}{3}$	$\frac{145}{3}$	$\frac{160}{3}$	$\frac{140}{-}$	$\frac{130}{-}$	$\frac{150}{4}$	$\frac{170}{2}$	$\frac{180}{2}$	17.0
July 16, 1975	$\frac{250}{6}$	$\frac{180}{4}$	$\frac{190}{3}$	$\frac{190}{4}$	$\frac{160}{2}$	$\frac{150}{1}$	$\frac{140}{16}$	$\frac{190}{3}$	$\frac{185}{3}$	20.0
July 31, 1975	$\frac{370}{10}$	$\frac{220}{6}$	$\frac{210}{5}$	$\frac{250}{8}$	$\frac{190}{3}$	$\frac{180}{3}$	$\frac{125}{17}$	$\frac{220}{4}$	$\frac{200}{4}$	19.0
August 15, 1975	$\frac{550}{13}$	$\frac{250}{6}$	$\frac{230}{6}$	$\frac{390}{10}$	$\frac{250}{3}$	$\frac{230}{3}$	$\frac{100}{18}$	$\frac{240}{5}$	$\frac{230}{7}$	21.0
August 30, 1975	$\frac{720}{18}$	$\frac{325}{5}$	$\frac{340}{5}$	$\frac{570}{13}$	$\frac{390}{3}$	$\frac{350}{3}$	$\frac{80}{20}$	$\frac{280}{6}$	$\frac{260}{6}$	22.0

fore the most numerous in the phyllosphere of urea treated *I. balsamina* and almost as numerous on *R. discolor*. The lowest number of fungi was always form in *L. rosea*. A promotive effect in former two species and inhibitory in the latter due to urea spraying was noted. No regular sequen-

ce in number of fungi in three plants in phylloplane of sprayed sets was exhibited except that the number of fungi in general increased gradually from July 1 to August 30, 1975. In all the cases fungi were considerably higher in urea sprayed sets than their respective controls. Maximum number of fungi on every sampling date was recorded in adjacent air (Table 2).

Except for urea-sprayed *L. rosea* where the fungal population gradually decreased with the age of the plant, it increased considerably in other cases. Use of urea enhanced the fungal population in phyllosphere of *I. balsamina* and *R. discolor*, however, in *L. rosea*. Its inhibitory effect was recorded as population in treated set was every time lower than its two controls. The population in phylloplane region recorded as number of colonies/plate was the highest in urea treated *L. rosea* followed by *I. balsamina* and *R. rosea*. The population in adjacent atmospheric air (calculated, as colonies per cm sq. area of nutrient surface of plate was much less than any of the phyllosphere (Table 3).

DISCUSSION

A large number of fungi were isolated from leaves of different sets which may be ascribed to the rich microflora of the adjacent air which settled over the leaves and many of them were germinated and parasitized the leaf tissues (Table 1).

Foliar application of urea enhanced the phyllosphere and phylloplane mycoflora both qualitatively and quantitatively in *I. balsamina* and *R. discolor* (Tables 1-3) which may be due to changed phyllosphere and phylloplane environments. The intake of urea in the above two plants supported the better growth of the foliage which in turn possibly resulted higher leakage of nutrients by leaves and provided more suitable conditions for the germination and colonization of fungi at their surfaces. Pretreatment of leaves of *Vicia faba* with substances that changed the permeability of the host plasma-membrane enhanced the susceptibility to attack by *Botrytis fabae* (Sol 1966). Foliar treatment of leaf by urea possibly changed the physiological behaviour of leaf and this may be held responsible for higher population and greater number of fungal species in rhizoplane region of *I. balsamina* and *R. discolor*. In *L. rosea*, however, the application of urea adversely affected the fungal population and number of fungal species in phyllosphere, whereas in phylloplane region the fungi increased both in quality and quantity (Tables 1-3). The health of the *L. rosea* itself was adversely affected by the use of urea. The treated plant grew pale and weaker as compared to controls which might have decreased the photosynthetic activity of plants, there-

by lesser exudation of nutrients was expected which in turn resulted in decreased population in this region. The moribund nature of leaf (Dickinson 1965; Mishra and Srivastava 1971, and Kanaujia 1975a) and possibly the loss in resistance caused quicker colonization by saprophytes and parasites of leaf.

Similarly to the rhizosphere, the phyllosphere is also affected by leaf exudates. The leaves from their surfaces exude nutrients like amino acids and sugars and other growth promoting substances. The amounts of exudation likewise vary in quality and quantity in plant to plant and even in the same plant at different environmental conditions (Dickinson 1965; Sol 1967, 1968 and Kanaujia 1975a, b). The common frequent distribution of few isolates in phyllosphere and phylloplane regions of the three plants, yet specific likings of the others are possibly related to the above statements.

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Studia nad grzybami fyllofery.

VII. Zastosowanie oprysku mocznikiem liści kilku roślin ozdobnych

Streszczenie

Badano wpływ oprysku 5% mocznikiem *Impatiens balsamina* L., *Rhoeo discolor* L. i *Lochnera rosea* (L.) Reichb. na rozwój mikoflory na powierzchni liści tych roślin. W pierwszych dwu przypadkach opryskiwanie korzystnie wpływało na rozwój mikoflory, podczas gdy w przypadku trzeciej rośliny efekt był przeciwny. Mocznik w zastosowanej koncentracji był toksyczny dla *L. rosea*.