OBSERVATIONS ON THE LIFE HISTORY OF THE RUST FUNGUS UROMYCES SPECIES ON JASMINUM MALABARICUM AND ITS RELATION TO UROMYCES HOBSONI VIZE. (U. CUNNINGHAMIANUS BARC.) ON JASMINUM GRANDIFLORUM

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Introduction.

In a paper "On the Identity of Blastospora (?) Butleri, Syd., Ajrekar (1) states that the rust fungus on Jasminum malabaricum is a true Uromyces species and not a Blastospora, as described by Sydow and Butler in their "Fungi Indiae Orientalis . Part IV. He further adds "that this Uromyces species on Jasminum malabaricum bears a very close resemblance to Uromyces Hobsoni, Vize (U. Cunninghamianus Barc.) on Jasminum grandiflorum. The teleuto and the aecidial stages agree closely on both the hosts in microscopic characters, as well as in the effects produced on the hosts. There is one striking difference, however, Whereas, U. Hobsoni has no uredo stage, as Barclay (2) has proved, the fungus on J. malabaricum shows a uredo stage in association with the teleuto. The writer made several inoculations and cross-inoculations with different sporeforms, found on either host but with negative results. It remains doubtful, therefore, if the fungi on the two hosts are identical and if the uredo stage found on J. malabaricum actually belongs to the Uromyces on it." The work recorded in this paper includes field observations, microscopic studies and inoculation-experiments carried out in order to throw light on this question.

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Field Observations.

Search was made in the neighbourhood of Bombay to collect the fungus material on both the hosts but it was not obtained there. The Jasminum malabaricum plants in the Canheri forests were free from any rust. Material of the fungus Uromyces Hobsoni was collected from Poona and Lonavala and that of rust fungus on Jasminum malabaricum was collected from Matheran (in December, 1927, April, 1928 and November, 1928). where it occurs in abundance. It was then possible to observe the rust fungus in natural conditions and in various stages of development and to draw certain conclusions as to the relation of the uredo stage to the aecidial and teleuto stages. The observations are summarised as follows:—

On all the occasions when the material was collected the uredo stage occurred prominently on young as well as old leaves. The sori generally occur on the lower side of the leaves in concentric circles (Plate I) but they were also seen on the upper side in a few cases. They were very rarely found on any other part of the plant. Owing to the presence of the fungus, dark brown spots occur on the upper surface of the leaf corresponding to the funguspustules on the lower surface. No hypertrophy or distortion takes place in this case. The aecidial stage (Plate I) occurred in abundance in the month of November, on leaves and young branches, causing hypertrophy and distortion. The sori were usually found on the lower surface of the leaves but in a few cases they were also seen on the upper surface. They were yellow in colour but this gradually gave place to a dark colour owing to the replacement of the aecidiospores by the teleuto spores. The older spots were, therefore, almost black in colour. The telento sori (Plate I) occurred more abundantly in December and still more in April. It was noted that both the aecidial and teleuto sori occurred more frequently on the stem than on leaves. This is in great contrast to the almost exclusive occurrence of the uredo stage on leaves and is one argument against the supposition that the uredo stage has any connection with the aecidial and the teleuto stages on this host (J. malabaricum).

Another observation made was that although the uredo sori and the aecidial and teleuto sori sometimes occurred side by side on the lower surface of the same leaf, they were never present in such close proximity as to sugrest any organic connection between them. The arrangement of the nredo sori in concentric circles which is so characteristic of the uredo on this host was never observed in the case of the aecidial and teleuto stares. The teleuto

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stage, moreover, was never found to be mixed up with the uredo (as was with the aecidio), as might be expected if the two had been genetically connected. The difference in the symptoms produced and the parts of the host chiefly attacked, the occurrence of the uredo sori side by side with the aecidial and teleuto sori when all the three stages occurred abundantly, the regular replacement of the aecidial stage by the teleuto and the entire absence of such replacement in the case of the uredo sori—all these point to the conclusion that the Uredo is entirely unconnected with the aecidial and teleuto stages and belongs to a separate rust fungus.

The previous observation by Barclay (2) that the uredo stage is omitted in the life-cycle of *Uromyces Hobsoni* which has also been confirmed in the writer's study, lends further support to this conclusion, because of the extreme similarity of the *Uromyces* species on the two hosts (Plates I & II).

Laboratory Study.

Microscopic studies.—Sufficient fungus material was collected when the field-observations were made and studied on the spot and also in the laboratory.

Material and methods.—Material in different stages was preserved in 4 per cent Formaline and used for free-hand sections which sufficed in many cases to learn the general structure of the fungi. Microtome sections were also taken and permanent slides prepared. The material used in this connection was in different stages of development and the methods were those suggested by Chamberlain (3). Chromoacetic acid, Fleming's weak, strong solutions, Juel's fluid, etc., were used as killing and Fixing agents. For embedding, paraffin of melting point $52^{\circ}-54^{\circ}$ C was used and sections were cut five to ten microns in thickness. Iron alum Heamatoxyline as included in Yamnouchi's schedule was used to stain the sections. Microscopic examination was made and the observations recorded as follows:—

I. Uredo on Jasminum malabaricum.—The Uredosori are hypophyllous in general and situated not very deep in the tissue of the leaf (Plate I). The stalked spores when fully developed are globose, echinulate and thick-walled. There are two prominent nuclei (conjugate) in the centre of the spore. The stalks which bear these spores as also the mycelium which gives rise to these are all binucleate (Plate I). No organic connection has been observed between the uredo and the teleuto sori and no teleuto

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spores were found in the uredosorus, as might be expected, had there been any genetic relation between the two kinds of spores.

II. Aecidio and teleuto sori on J malabaricum (uromyces sp.) (Plate I). The sori occurred on both sides of the leaf and no spermogonia were seen. This might be due to their early disorganisation as noted by Barclay (2) in the case of U. Hobsoni. The aecidiospores and the teleutospores which occur in the same sori, as also the mycelium which bears these spores are all unimu eleate, as far as the writer's observations go. But this point requires further careful study.

III. Uromyces Hobsoni on Jasminum grandiflorum. -(Plate II). The sori occurred on both the sides of the leaf and no spermogonia were seen due to the probable cause mentioned above.

The aecidiospores and the teleutospores which occur in the same matured sori, as also the mycelium which produces the teleutospores and the hyphae from which the interealary cells and the aecidiospores are abstricted are all binucleate (Plate II, Figs. 7, 8, 14 No nuclear migration from one cell to another in a young aecidiosorus was observed, although, the presence of uninueleate mycelium at the base of a mature sorus leads one to believe that the migrations must have taken place to attain the binucleate conditions, from uninucleate one. Whether this is a constant difference between the two Uromyces species under consideration and whether this points to their being distinct varieties, if not species, of Uromyces is at present doubtful.

Gwynn Vaughan (5) suggests that the cytology of such a rust rungus as Uromyces Hobsoni Vize would be interesting since it would probably present the observer with an aecidiosorus which may be different from a typical one because this contains an already binucleate mycelium which is produced from a binucleate aecidiospore. No fusion of cells or migration of nuclei would be necessary in such cases. The writers have not been able to study this point, although it is an important one.

Germination—Studies and inoculation—Experiments.—The most conclusive evidence bearing on the question of the identity of the rust fungi occurring on different hosts or on the genetic connection between their different spore-forms, is that obtained from inoculation experiments, especially if positive results are obtained. Some inoculation and cross-inoculation experiments were, therefore, carried out with the various sporeforms under consideration and these are presented in Table-No. 11 It must be said that these inoculation experiments were not entirely satisfactory, for the reason that germinating spore

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material was not available for experiments in most cases. They are not, therefore, regarded as conclusive.

Material and methods.—When the field-observations were made, sufficient spore-material in different stages of development was collected and stored in paper envelopes for use in the experiments. Before inoculation experiment was made it was first determined whether the spores were in a germinating stage, by spreading at few spores on the surface of distilled water in a watch-glass as suggested by Plowright (7) and Grove (4). These watch-glasses were at times kept in darkness and light alternately. They were also at times kept in water in a Petri-dish-jacket lined with moist blotting paper and the medium was changed at times to 5% sugar solution or Potassium di-hydrogen Phosphate (5% aqueous solution) as suggested by Maneval (6). Microscopic examination of the contents in the watch-glass was made daily and the results were recorded as follows in Table No. I.

TA	BLE	N	0.	1.

No.	Date of starting.	Kind of spores.	Conditions of germination.	Results.
1	26th June, 1927.	Teleuto on J. grandiflorum.	Watch-glass with distilled water.	No germi- nation.
2-400	-		_	
401	5th Jan., 1929.	Teleuto of U. Hobsoni Lonavla, May, 1927.	Do.	Do.

Experiments in Spore-germination.

(The date and the place mentioned in the 3rd Column indicates the date and place of collection.)

It was seen from the observations that the teleutospores fresh as well as those collected even at different times very rarely germinated under the laboratory conditions. It is clear, therefore, that they do not germinate when they are fresh; but nothing can be said as regards the resting period they undergo before they begin to germinate. In January, 1928, the germination of the aecidiospores of *Uromyces Hobsoni* was observed and this took place after the manner described by Barclay (2). In February, 1928, the germination of the teleutospores of both the *Uromyces species* was seen but only then, and not later—(NGs. 8. 10,

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12). In November, 1928, however, the germination of the three kinds of spores on Jasminum malabaricum was seen to take place at Matheran (Nos. 72-76). The uredospores germinated in less than 4 hours and put forth a long, unseptate and thick germ tube through the terminal germ-pore. The teleutespores (very few) germinated in the usual way described by Ajrekar (1) in the case of Uromyces species on Jasminum malabaricum and by Barclav (2) in the case of Uromyces Hobsoni The aecideospores of Uromyces on Jasminum malabaricum germinated in a way different from that described by Barclav (2) in the case of Uromyces Hobsoni Viz. Whereas, Barclay (2) states that in the case of Uromyces Hobsoni, each of the two cells of the germ tube of the aecidiospore bears one sterigma each, the lower cell having a lateral and the upper cell having a terminal one. in the case of Uromyces species on Jasminum malabaricum it is seen that only the upper cell of the two-celled germ tube bears a terminal storigma (Plate I). This difference in the mode of germination can be taken as an evidence to support the conclusion that the two Uromyces species in question are not the same

It was found that such spores when brought to Bombay did not germinate, as also those brought from Mount Abu

In the absence of germinating material, non-germinating spores were also used in some of the inoculation experiments and these also gave negative results, as will be seen from Table No II.

Date of inoculation.	Kind of spores.	Number of plants.	Results.
15th Dec. 1927.	Teleuto on J. malabaricum fresh.	I. J. Malabaricum.	No infection Control Healthy.

	TABLE NO. II.	
Showing	the inoculation experiment	13

..... Etc., Etc., Etc.

(26 observations in this Table further, omitted.)

The uniformly negative results obtained in the cross inoculations between J malabaricum and J grandiflorum might have been regarded as pointing to the two I romyces species being distinct biologic forms; but much stress cannot be laid on this negative evidence in view of the fact that inoculations on Jasminum granditiorum with its own rust have also failed in all cases except in a solitary one,

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Descriptions of the Rusts.

1. Uramyces Hobsoni Vize (Uromyces Cunning. Barc.) on Jasminum grandiflorum Linn. (Plate II).

Aecidiosori.—Hypophyllous, rarely amphigenis, aggregate in variously shaped patches, mixed with teleutosori; consisting of periodial cups. Deep sellow in colour.

Teleptonori. Formed by the replacement of accidiospores by teleptonories dark-brown to almost black in colour. Both kinds of sori found on leaves, petioles and stems.

Aecidiospores.-Thin-walled, globose or round, orange yellow in colour

Teleutospores.—Thick-walled all over but more thickened at the top, one-celled mostly ovate in shape, brown m colour, apex $20.44 \times 19-24 \mu$ Pedicel deciduous and trainally situated. Except in the mode of germination of aecidiospores, this rust is identical with 11 Uromyces species on Jasminum malabaricum.

Details of germination are given in Table I; Fungus studied by Barelay (2) and reported to occur at Simla and Sairi.

Collected at Poona, Lonavla and Mount Abu in October, November, December, April, May.

2. Uromyces species on Jasminum malabaricum Wight. (Pl. No. I) Aecidiosori and teleutosori similar to those described above (No. 7). Details of germination-studies given in Table 1.

Collected at Matheran in October, November, December, etc. Rust-fungus studied by Ajrekar (1).

3. Uredo on Jasminum malabaricum Wight. (Pl. No. I).

Uredosori.—Hypophyllous rarely amphygenis, erumpent, solitary or aggregated in concentric circles, orange-yellow in colour.

Uredospores.—Thick-walled, minutely spiny, globose or ovate in shape, orange-yellow in colour; epispore $35-39 \ \mu \times 20-25 \ \mu$. Spore $16-20 \ \mu \times 28-29 \ \mu$ Pedicel hyaline, deciduous. There is only one germ-pore terminally situated as already stated.

Collected at Matheran only, during the months of November, December, April.

4. Uredo on Jasminum pubescence (?) Willd (Pl. II).

Uredosori.—Hypophyllous, erumpent, solitary, round, scattered and also arranged in concentric circles, orange-yellow in colour. 26-33 $\mu \times 16-22 \mu$. Epispore 30-36 $\mu \times 18-24 \mu$. Pedicel hyaline, deciduous.

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Germination of spores tried 5 days after collection but with negative results (Table 1). Fungus resembles the Uredo on Jasminum malabaricum, (described above) both in the symptoms produced on the host as also in Microscopic characters.

Collected at Mt. Abu by Ajrekar in December.

Summary and Conclusions.

I. Field-observations and microscopic studies support the conclusion that the Uredo on *Jasminum malabaricum* is in no way connected with the Uromvces species on the same host. It is a distinct rust fungus occurring in the uredo stage only This conclusion is also supported by the study of a similar rust fungus occurring in the uredo stage on *Jasminum pubescence* (?) Willd. 1 Mt. Abu.

II. Field-observations, microscopic studies, germinationstudies and inoculation experiments all support the conclusion inat the two Uromyces species in question are not identical.

III The germination-studies have shown that the germination of the teleutospores of Uromyces species on J grandifierum and J malabaricum very rarely took place under the laboratory conditions m Bombay. That these spores do not germinate at once but require probably a long resting period. The aecidiospores and the uredo spores germinate readily when they are mature and fresh as can be seen from the experiments on germinations, made on the spot and in the laboratory.

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THE ROYAL INSTITUTE OF SCIENCE, BOMBAY, February, 1929 Explanation of Plates I-II.

Plate	No. I :- I	Rust fungus on Jasminum malabari	cum.
	Fig. 1. 8	Shoot of J. malabaricum with aecid	lio, teleuto and
		uredo-sori. Natural Size.	
		u-uredosori: A-Aecidiosori; T-Te	eleutosori.
	Fig. 2. I	ongitudinal Section of Accidium Peridium; S-Spores.	showing P- (15×8) .
	Fig. 3. I	long. Sec. of Teleutosorus	27
	Fig. 4.	" " young uredosoru	5
	Fig. 5.	a Free uredospores	
	-	D ,, ,,	(15×4 0)
	Fig. 6.	a ,, teleutospores	(15×8).
		Ъ ,, ,,	(15×40)
	Fig. 7.	" aecidiospores	$(15 \times 40).$
	Fig. 8.	Part of an uredosorus on J. ma	labaricum with
		S-young spore; H-Hyphae-sho	wing conjugate
		nuclei	(15×90).
	Fig. 9.	A-aecidiospore; St-sterigma; G-	-germ-tube; E_
		endochrome. (J. malabaricum).	Diagramatic
	Fig. 10,	T-teleutospore; P-promycelium	; 12
	Fig. 11.	U–uredospore; G–germ-tube.	"
	Fig. 12.	A-aecidiospore; G-germ-tube;	St-sterigmata,
(J.	grandifion	cum),	
Diata	No. II.	Thomas Hobsoni on January	
rate	NO. 11;-	theat of I wand difference with a	granainorum.
	rlg. 1. 7	tosori. Natural Size.	eldio and teleu-
	171:	A-Accidiosoff, 1-reletitosoff;	(15 \ \)
	Fig. 2.	Long. Sec. of Accidition	(10^8).
	Fig. 5.	, , , relettosorus	"
	Fig. 4.	y Free and young teleutospore	(15×40)
		a Free and old teleutospore	
	Fig 5	Free aecidiospore	<u>**</u>
	Fig. 6.	neridial cell	37
	Fig. 0.	Part of the applidiosorus of T	Hohsoni on I
	r 1g. 1.	arandiflarum	(15×90)
		A-hinucleate aecidiospore	M-aecidiospore-
		mothercell; I-intercalary cell;	accurtoshort.

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Fig.	8.	P-peridial cel.	$(15 \times 90).$
Fig.	9.	L-lower side of the leaf	of Jasminum pube-
		scence, with sori. Natural	Size

Fig. 10. S-1 redosporus in Long sec. (15×8) .

Figs 11-13. U-uredospores

Fig. 14 Binucleate mycelium M of l romyces Hobsoni on J. grandiflorum with the young teleutospore Γ in the top. (15×90) .



J. L. B. S. X; 3.

PLATE II.



J. I. B. S. X : 3.