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Role of aeciospores in outbreaks of pea (*Pisum sativum*) rust (*Uromyces fabae*)

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Abstract

Aeciospores in *Uromyces fabae* were found to be repeating spores and play an important role in pea rust outbreaks in the North Eastern Plain Zone (NEPZ) of India. Experiments conducted on pea rust from 2001 to 2004 revealed the dominance of aeciospores at all growth stages of pea in this region. Urediospore production was erratic and was only observed in a few samples of stems and tendrils (5–10%). Inoculation of pea plants either by aeciospores or urediospores resulted in the production of aeciospores. Production of aeciospores was observed at a temperature range of 10–25 °C, with a maximum at 25 ± 2 °C. Among the different growth stages of pea, the pod formation stage was highly susceptible and produced the maximum number (744) of aecidia/leaf at 20–25 °C. Significant effects of growth stages and temperature were also noticed for pustule number. Urediospore production mainly coincided with the senescence of the pea plants. Maximum germination (2%) of aeciospores was observed at 25 °C, whereas maximum urediospore germination (3.5%) was at 15 °C. Temperatures >15 °C decreased urediospore germination. A relative humidity (RH) of 100% was favourable for aeciospore germination while 98% RH favoured urediospore germination. Typical histo-pathological behaviour of the aeciospores was observed.

Introduction

Typical macrocyclic rust fungi have all the five distinct spore stages *i.e.* spermatia, aeciospores, urediospores, teliospores and basidiospores. Aeciospores are the first dikaryotic spore stage of the rust fungi. In a few hosts, aeciospores act as repeating spores and behave like urediospores. Cummins (1959) used the term 'uredinoid aecidium' for such spore stages. In such genera, a distinction between the aeciospore and urediospore is sometimes difficult to make. In general, teliospores help in the survival and the generation of genetic variability in rust pathogens (Boehm et al., 19922), while the asexual life cycle promotes multiplication and dissemination (Singh, 1999). A particular spore stage that repeats itself causing further infection during the cropping season is referred to as a repeating spore; these play a significant role in the secondary infection cycles during disease development and polycyclic epidemic outbreaks. A large number of asexual cycles also increases the chances for selection of more aggressive strains and allows for their multiplication. This leads to the build-up of more aggressive populations and increases the chances of an epidemic outbreak under favourable conditions.

For *Uromyces fabae*, an autoecious rust, both aeciospores and urediospores are found on the same plant. Rust outbreaks are responsible for significant yield losses in peas (*Pisum sativum*), especially in the sub-tropical regions of the world characterized by warm humid weather conditions (Kumar et al., 1994). These conditions usually

coincide with the reproductive phase of the pea crop and favour the outbreak of the disease (Chand et al., 2006). Basic information related to the biology of aeciospores and their role in the outbreak of rust is meagre or lacking. Therefore, the present investigation was undertaken to generate information about important spore stages and their role in disease outbreaks to develop effective long-term disease management strategies.

Materials and methods

Natural occurrence of different stages of rust on pea

Six genotypes of pea *viz*., HFP 4, Rachna, HUVP 1, FC 1, Pant P 11 and S 143 were planted at five different locations in farmers fields in plots of 5×3 m^2 in 50 km periphery of Banaras Hindu University, Varanasi, India from 2001 to 2004. Fifteen infected pea plants of each genotype from all the five locations were collected every year, and examined for the type of spore stages of rust at pod formation stage. Number and type of pustules on leaves, stems, and tendrils on 150 different infected plants were recorded as percentage of the total number of pustules/plant.

Effect of urediospore and aeciospore inoculation on production of the spore stages

The experiment was conducted on the same six genotypes of pea as mentioned above in two separate polyhouses with partial temperature and humidity control; plants were inoculated either with aeciospores and urediospores in a randomized block design with three replicates. Five plants of each genotype in one pot constituted one replicate. For field experiments, three rows of each genotype were sown with a row-to-row and plant-to-plant distances of 45 and 15 cm, respectively; plants of each genotype were sown at two locations separated by a distance of 500 m. Leaves with typical aecidia (obtained by single pustule multiplication on the susceptible cv. HFP 4, Figure 1a) were soaked in water for 5 min and agitated vigorously to dislodge the aeciospores. Similarly, the plant parts showing typical brown to black uredia (Figure 1b) were also soaked in water and agitated. The spore suspension was filtered through cheesecloth and adjusted to 10^4 spores ml^{-1}. Spores were verified under the light microscope for morphological features. In the polyhouse and the field, plants 40–50 days after sowing (DAS) in the vegetative stage were inoculated to run-off either with aeciospore or urediospore suspensions using hand sprayers (Chand et al., 2004; Vijayalakshmi et al., 2005). After inoculation a sprinkler was run for 15 min to maintain free water on the leaf surface, and the fields were irrigated immediately after inoculation to maintain high humidity for the experiments. Number of aecidia/leaf was recorded at 20 days after inoculation and the spore type was confirmed under the light microscope. Comparison of means was done by LSD ($P < 0.05$).

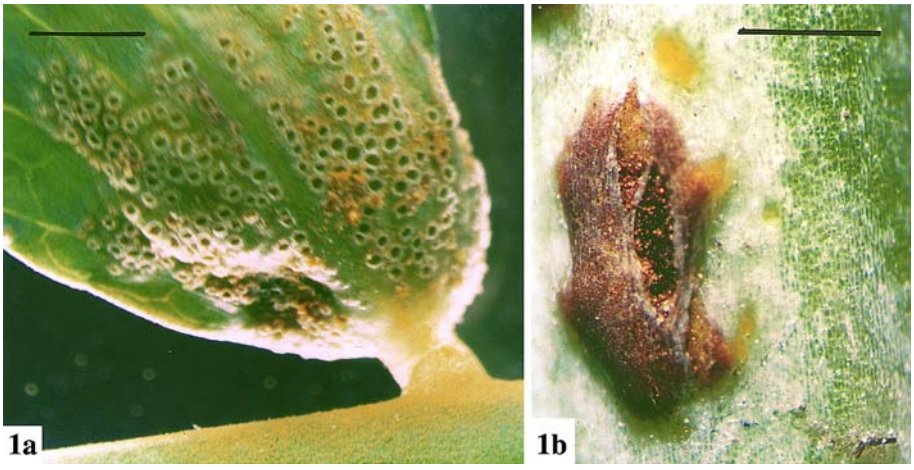

Figure 1. (a) Typical aecdial infection on leaf. (b) Typical uredial infection on stem. Bars a = 5000 μm, b = 2000 μm.

Effects of growth stage of pea on spore production at different temperatures

Two temperature regimes 10–15 and 20–25 °C were created artificially in two different polyhouses. The seed of pea cv. HFP 4 was sown at different dates in order to synchronize the various growth stages *i.e.* seedling (10–30 DAS), vegetative (30–50 DAS), pre-flowering (50–70 DAS), flowering (70–90 DAS) and pod formation (90–110 DAS). Three rows for each of the growth stages were raised and a minimum of 10 plants per row was maintained. Sets of different growth stages were raised in each of the polyhouses. The experiment was conducted in a randomized block design for both the temperature regimes. Inoculation with aeciospores was done as mentioned above. Number of aecidia/leaf was recorded 20 days after inoculation. Five plants were chosen randomly from each row and assessed; this represented one replication. Analysis of variance (ANOVA) was done for the replicated data generated.

Effects of relative humidity (RH) and temperature on aeciospore, urediospore and teliospore germination

A set of five different RH chambers (100, 98, 95, 92 and 88.5%) were prepared by adding 0.0, 0.3, 0.6, 1.5, 2.2, and 3.1 M solution of NaCl in 2% water agar as described by Lang (1967). Suspensions of aeciospores, urediospores and teliospores were prepared from 3 day-old ruptured pustules. One drop (300 μl) of spore suspension (10^4 spores ml^{-1}) was placed on the centre of a grease-free slide and then air-dried. Two slides each were placed in each of the RH chambers and incubated at temperatures of 5, 10, 15, 20 and 25 °C in the dark. Therefore, there were 25 treatment combinations. Germination of spores was recorded at 72 h. Spores were observed at a 250× magnification on a compound microscope (area of the microscopic field was 1 mm^2). Percent germination was based on a count of five times 200 spores/slide. The mean of six slides (in three chambers under a single treatment combination) constituted one replicate; thus a total of 6000 spores were examined in each replicate. A spore was considered to be germinated when the germ tube was as long as the diameter of the spore. The germination of teliospores was investigated using a range of techniques (Anikster, 1986; Bruckart and Eskandari, 2002). The teliospore suspension was placed on 2% water agar and incubated at 15, 20, and 25 °C for 20 days. Similarly, germination was investigated at various concentrations of pea leaf extract and soil extract. The experiment was conducted three times in a factorial randomized block design with temperature as the first factor and RH as the second. ANOVA was done with the Tukey test at $P < 0.05$.

Effect of plant debris on initiation of infection

Plant materials bearing telia from the previous season were stored at 4 °C; 25 g of this material were mixed in the upper soil in each of the pots (30 cm diam). Ten pots with plant debris and 10 pots without debris were sown with pea seeds of susceptible genotype HUVP 1. Pots without plant debris served as the control. Pots were watered 7 days prior to sowing of pea seeds. Five plants per pot were maintained and placed in the polyhouse. Observations were taken on the appearance of spermatogonia, initiation of the disease and appearance of the spore stages.

Histo-pathological characteristics of different spores

Leaves, tendrils, and stems bearing aecidia, uredia and telia were collected from the cv. Rachna for histo-pathological examination. Free-hand sections of these specimens were cut, mounted in 1.0% lactophenol and stained with cotton blue. Sections were observed at 250× magnification under the light microscope. The morphology of the aecidia, uredia and telia was studied and photomicrographs taken.

Results

Natural occurrence of different stages of rust on pea

The natural occurrence of aeciospores and urediospores during 2001–2004, from vegetative to pod formation stages of pea averaged over these years is presented in Table 1. Occurrence of urediospores varied from 5.3 to 7.4% in the samples and was often restricted to stems and tendrils. Aeciospores were found at all growth stages of the crop and were generally confined to leaves occu-

Table 1. Proportion of different spore stages produced on six different pea genotypes averaged over 2001–2004

Genotype	Aecidia (%)/plant	Distribution	Uredia (%)/plant	Distribution
HFP 4	92.8 ± 0.7	Stipules and tendrils	7.3 ± 0.8	Stem and tendrils
Rachna	94.7 ± 0.3	Leaf	5.3 ± 0.3	Stem
FC 1	92.6 ± 0.4	Leaf	7.4 ± 0.5	Stem
Pant P 11	93.6 ± 1.3	Leaf	6.3 ± 1.5	Stem
HUVP 1	94.4 ± 1.5	Leaf	5.6 ± 1.8	Stem and tendrils
S 143	94.0 ± 1.7	Leaf	6.0 ± 2.1	Stem
Mean	93.7 ± 0.5		6.3 ± 0.6	

pying more than 90% of the leaf area. Teliospores were formed in the uredia at the time of senescence.

Effect of urediospore and aeciospore inoculation on the production of aecidia

Formation of aecidia after artificial inoculation with aeciospores and urediospores was observed on all six genotypes of pea under polyhouse and field conditions (Table 2). The observed numbers of aecidial pustules on different genotypes in the polyhouse were comparable to those in the field and there were only small changes in the ranking order of the test genotypes.

Effects of growth stage of pea on spore production at different temperatures

The higher temperature promoted considerably high numbers of aecial pustules than the lower temperature. Teliospores were produced when plants reached the senescence stage (110–130 DAS). Aeciospores were produced abundantly at both temperature regimes, 10–15 and 20–25 °C at all growth stages (Table 3). At both temperature regimes, the number of aecidial pustules/leaf was highest at the pod formation stage. Significant effect of growth stages was observed on number of pustules/leaf at the lower temperature regime (Table 4).

Effects of RH and temperature on aeciospore, urediospore and teliospore germination

The optimum condition for aeciospore germination was 25 °C in combination with 100% RH (Table 5). The % germination of aeciospores decreased gradually with the decrease in temperature below 25 °C. None of the aeciospores germinated at a RH of 88.5% at any of the temperature regimes studied. Aeciospore germination started at 5 °C in combination with 100% RH (0.17%) and peaked at 25 °C with 100% RH (2%). RH of 98% in combination with 15 °C favoured maximum (3.50 %) urediospore germination (Table 6). The interaction between temperature and RH on the germination of aeciospores and urediospores was significant

Table 2. Aecidial production after inoculation with urediospores or aeciospores on plants of six pea genotypes grown either in the polyhouse or field

Genotype	Mean number of aecidia/leaf formed on inoculation with aeciospores		Mean number of aecidia/leaf formed on inoculation with urediospores	
	Polyhouse	Field	Polyhouse	Field
HFP 4	64^{b}	51^{b}	102^{bc}	104^{bc}
Rachna	84^{b}	83^{b}	83^{b}	85^{b}
FC 1	34^{a}	48^{a}	17^{a}	17^{a}
Pant P 11	18^{a}	20^{a}	14^{a}	15^{a}
HUVP 1	211^{d}	205^{d}	138^{c}	135^{c}
S 143	130^{c}	136^{c}	101^{bc}	104^{bc}
LSD (0.05)	42.5	48.6	53.9	57.2

Values with letters in common are not significantly different ($P < 0.05$, LSD test).

Table 3. Effect of growth stage on formation of aecidia on pea cv. HFP 4 at two temperature regimes

Growth stage	Mean number of aecidia/leaf Temperature (°C)	
	10–15	20–25
Seedling	157[a]	504[a]
Vegetative	295[ab]	550[ab]
Pre-flowering	317[bc]	663[abc]
Flowering	319[c]	715[bc]
Podding	402[c]	744[c]
LSD (0.05) growth stage	138.9	172.4

Values with letters in common are not significantly different ($P < 0.05$, LSD test).

(Table 7). No teliospore germination was observed under any of the experimental conditions.

Effects of plant debris on initiation of infection

The disease appeared at the late vegetative phase (50–60 DAS). No spermatogonia were found on plants and disease was observed as aecidia on the lower surface of the leaves from which infection spread to the upper leaves. Initial symptoms appeared in as few as five plants out of 50 raised under infected soil. No symptoms were observed on the control plants.

Histo-pathological characteristics of different spores

Observation of free-hand sections of the leaves, stems and tendrils revealed that aecial cups extended into the spongy parenchymatous cells of the leaf (loosely arranged mesophyll cells). Uredinia were confined between the epidermal and parenchymatous cells (Figure 2a, b).

Discussion

In the present investigation, aeciospores were associated with all growth stages of the pea plant and served as the repeating spore during 3 years of testing. This was confirmed by the appearance of aecidia subsequent to inoculation with the aeciospores. Aecidia were confirmed by histopathological studies. For such a catenulate repeating vegetative spore stage, the term 'uredial aecia' was coined by Hiratsuka (1973). Similar observations have also been made in *Puccinia senecionis*, *Uromyces hedysari-obscuri* and *Phragmidium disciflorum*, where aecia are repeatedly produced on binucleate mycelium arising from the germinating aeciospores (Gäumann, 1998). Generally, the urediospore is the repeating spore in most of the rust species and those forms in which binucleate mycelium is produced by the aeciospore to form more aecia are the exception (Gäumann, 1998).

Table 4. Analysis of variance for effect of growth stage on formation of aecidia on pea cv. HFP 4 at two temperature regimes

Sources of variation	df	Temperature (10–15 °C)			Temperature (20–25 °C)		
		MS	*F* value	*P*	MS	*F* value	*P*
Replicates	2	8820.6	1.62		24764.6	2.95	
Growth stages	4	23482.5	4.32	0.037	32825.1	3.92	0.65
Error	8	5441.35			8385.1		

Table 5. Effect of temperature and relative humidity on aeciospore germination

Temperature (°C)	Relative humidity (%)				
	100	98	95	92	88.5
	Mean aeciospore germination (%)				
5	0.17[ab]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
10	0.33[abc]	0.33[abc]	0.17[ab]	0.00[a]	0.00[a]
15	1.33[cd]	1.00[abcd]	0.33[abc]	0.00[a]	0.00[a]
20	1.33[cd]	0.67[abc]	0.50[abc]	0.33[abc]	0.00[a]
25	2.00[d]	1.00[abcd]	0.67[abc]	0.33[abc]	0.00[a]
HSD (0.05) Temperature × humidity				1.09	

Values with letters in common are not significantly different ($P < 0.05$, LSD test).

In *Uromyces fabae*, urediospores have been reported to be the repeating spore in cool temperate regions (Xue and Warkentin, 2002). However, the NEPZ of India is characterized by warm humid weather with mean temperatures > 17 °C during the cropping season, which is suitable for both aeciospore germination and aecidial formation. The most favourable conditions for aeciospore germination are at 25 °C in combination with 100% RH. Since low temperatures of around 15 °C occur rarely at pod formation in this region; this may be one of the reasons why urediospores are produced only occasionally under the warm humid conditions. As higher temperatures also promote the formation of more aecidia (Table 3), the climatic conditions further enhance the dominance of the aecidial state. Under these conditions, early sowing of pea genotypes would be helpful in reducing the disease severity and such practices may be an important component of integrated disease management.

The percentage of uredial formation was very low and they were mostly found on stems and tendrils whereas aecidia were mostly found on leaves. The differences in the topography of the leaves and the stem surfaces may result in the differential production of aeciospores and urediospores on particular organs. The pea genotypes chosen for this study differed conspicuously in their leaf morphology. HFP 4 is an afila genotype with normal sized stipules; S 143 has the pleifila phenotype where the leaf consists of tiny leaflets in contrast to the conventional leaf. Further, the stipule size is also reduced considerably as compared to normal stipules. HUVP 1 is the tendrilless (acacia) genotype and has normal sized stipules. FC 1, Pant P 11 and Rachna are normal foliaged genotypes with 3–4 pairs of leaflets and the terminal leaflets are converted into tendrils. The appearance of uredia was highest on HFP 4 when compared to HUVP 1 whereas both types were susceptible to *U. fabae* and showed high total numbers of pustules/plant. Differences in disease responses may therefore occur when inoculated by aeciospores or urediospores. Differences in the resistance response were also reported in beans when inoculated by aeciospores or urediospores (Groth, 1988). Therefore, screening for resistance may differ under conditions where aeciospores are causing the disease, particularly under warm humid conditions.

In the present study teliospores were not found to be germinating and no spermatogonia were observed. The appearance of symptoms on plants

Table 6. Effect of temperature and relative humidity on urediospore germination

Temperature (°C)	Relative humidity (%)				
	100	98	95	92	88.5
	Mean urediospore germination (%)				
5	1.83^{cdefg}	2.22^{defg}	1.17^{abcdef}	0.83^{abcd}	0.00^{a}
10	1.00^{abcde}	2.83^{fg}	0.33^{abc}	0.33^{abc}	0.00^{a}
15	2.00^{cdefg}	3.50^{g}	0.67^{abcd}	0.33^{abc}	0.17^{ab}
20	1.67^{abcdef}	2.67^{fg}	1.17^{abcdef}	1.00^{abcde}	0.00^{a}
25	0.00^{a}	0.17^{ab}	0.00^{a}	0.00^{a}	0.00^{a}
HSD (0.05) Temperature × humidity					1.78

Values with letters in common are not significantly different ($P < 0.05$).

Table 7. Analysis of variance for effects of temperature and relative humidity on germination of aeciospores and urediospores

Sources of variation	df	Urediospores			Aeciospores		
		MS	*F* value	*P*	MS	*F* value	*P*
Replicates	2	0.86	2.99	0.059	0.21	1.86	0.166
Humidity	4	11.27	39.38	< 0.0001	2.53	22.43	< 0.0001
Temperature	4	4.45	15.45	< 0.0001	1.47	13.05	< 0.0001
Humidity × temperature	16	0.93	3.24	0.0008	0.29	2.59	0.006
Error	48	0.29			0.11		

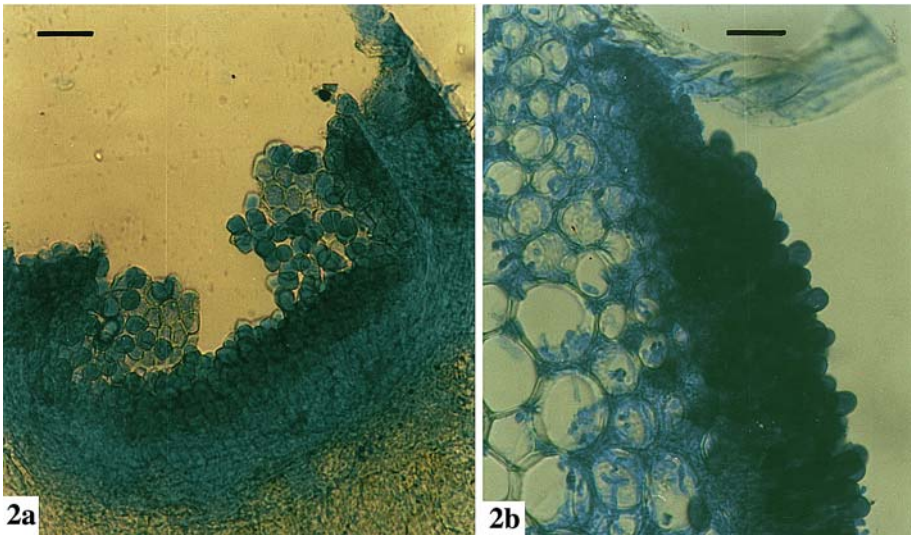

Figure 2. (a) Transverse section of aecidia on leaves. (b) Transverse section of uredia on stem. Bars a and b = 40 μm.

raised with plant debris indicates however, that there is a role of teliospores in initiating the disease. Rare occurrences of spermatogonia have been reported in *U. fabae* (Kapooria and Sinha, 1966, 1971). Binucleate basidiospores are a common feature in *Uromyces* and have also been reported in *U. viciae fabae* (Anikster, 1983; Freytag et al., 1988). Under such conditions the usual occurrence of spermatogonia may not be expected. The appearance of aecidia subsequent to inoculation by urediospores is an unusual event and may be due to environmental conditions which promote development of aecidia at higher temperatures. This aspect needs to be investigated in more detail. The present work reveals that aeciospores play an important role in pea rust epidemiology under warm humid conditions and may be of significance in screening for rust resistance.

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