Microscopical Studies on Interactions of Host and Non-host Plants with Bean Rust (*Uromyces viciae-fabae* (Pers.) Schroet)

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Abstract

Microscopical investigations were made to examine the interaction between host and non-host plant species inoculated with *U. viciae-fabae*. A tissue clearing method was used by overnight exposure of about 1 cm^2 cut inoculated leaf tissue in 95% methanol followed by 4-6 hour in 0.25% chloral hydrate alone and with added Trypan blue. A comparison of infection of hosts and non-host plants by the pathogen showed significant variation ($p < 0.01$, $df = 13$) in the behavior of the fungus on leaf surfaces and there was also a clear difference in intercellular growth. In most non-host and resistant hosts fewer germ tubes have located stomata due to reduced directional growth. From the few located some hyphae were successful in penetrating through the stomata. Among the tested materials, faba bean and pea cvs Kelvedon Wonder, Feltham First and Waverex showed susceptible reactions resulting in sporulation. In contrast to susceptible and resistant hosts, many hyphae stopped growing in leaves of non-host at early stage of intercellular development, except in White clover where the hyphae continued to grow to the stage of haustorium formation. In resistant hosts, except Tufted vetch, the intercellular hyphae continued to grow to the stage of first haustorium formation and sporulation in sweet pea cvs and partially resistant pea cv. Greenshaft, respectively. All resistant plant species showed the hypersensitive response at different stages of disease development. The hypersensitive response of guard cells, epidermal cells, mesophyll cells, and deposition of induced phenolic substances on the walls of infection hyphae and accumulation of such materials on the plant cell walls of the attempted penetration site appeared to restrict further growth of the fungus in plant species showing resistance. The restricted sporulation of this particular isolate of *U. viciae fabae* in only *Viciae faba* and *Pisum sativum* indicates that it has very narrow host range. This in turn indicates that this isolate has very little importance than the one known to infect large number of hosts.

Introduction

Faba bean rust, caused by *U. viciae-fabae* (Pers.) Schroet) is an important disease of faba bean and other related legumes wherever these crops are grown throughout the world. Efforts have been made to tackle the problem by developing resistant varieties for many years but the short duration of the resistance governed by few major genes requires constant screening of varieties against newly emerging races of the pathogen (Bernier and Conner, 1982). In addition to this, it seems feasible to introduce non-host resistance into the host plants using modern molecular biology techniques to develop durable disease resistant cultivars. In order to utilise this type of resistance mechanism, it is necessary to have a proper understanding on infection development of the disease, its host range and the mechanisms involved in the resistance responses.

Important ultrastructural studies of interactions between rust fungi and plants were carried out by Heath and Heath (1971). They examined susceptible and immune reactions of *Vigna sinensis* leaves infected with *Uromyces phaseoli* var *vignae*. They found that incompatibility was expressed in the immune variety during early stage of haustorial growth when a deposit of callose-containing material was formed on the host cell wall around the point of entry of the haustorium. No such reaction was observed in a susceptible variety. Haustorium formation in the immune variety resulted in either the simultaneous collapse of both host cell and haustorium or the enclosure of the living haustorium in a callose-containing sheath.

Heath (1972) in host and non-host plants inoculated with cowpea rust, *Uromyces phaseoli* var. *vignae* found that non-host, *Phaseolus vulgaris*, responded to each infection hypha by the deposition of electron-opaque material on and within the surrounding host cell walls. These deposits prevented haustorium formation at infection sites. In the host immune varieties she found similar results. In both the resistant host and non-host, death of haustorium and the haustorial mother cell did not result in the immediate death of the intercellular mycelium. She suggested that starvation was the primary cause of the cessation of fungal growth.

Heath (1974) further indicated that urediospores of *Uromyces phaseoli* var. *vignae* usually germinate equally on hosts and non-hosts. On most of the non-hosts, germ-tube rarely contacted stomata because of the reduced efficiency of directional growth. Compared with both resistant and susceptible host cultivars, infection hyphae in non-host leaves stopped growing during the very early stage of intercellular development. Nevertheless, the majority of the hyphae continued to grow to the stage at which the first haustorial mother cell would be expected to form. Based on these studies, she suggested that three mechanisms are involved in the inhibition of haustorium formation. These are: deposition of osmiophilic material on adjacent non-host walls in *Phaseolus* spp., loss of contact between haustorial mother cell and non-host cell in *Vicia faba*, or fungal death before haustorium initiation in *Pisum sativum*. In non-hosts if a haustorium was formed, rapid death of both haustorium and invaded cells was observed. In immune cowpea cultivars she observed the deposition of callose containing material near infection hypha or around the site of penetration. While in resistant cultivars there was retardation in the fungal growth by slow disorganisation of the plant cell membrane before sporulation.

The diversity of cellular response of host and non-host plants to cowpea rust fungus enabled to suggest that there are a number of stages during infection at which an interaction between this fungus and the higher plants take place (Heath, 1974). Based on these ultastructural observations Heath developed a scheme of events that occurs during the infection process that could lead to the observed resistance or susceptibility of host and non-host plants.

Similar studies, between the interaction of resistant and susceptible varieties of wheat, and non-host species with *Puccinia graminis tritici* identified three groups of varieties: resistant, intermediate and susceptible on the basis of colony growth and the amount and proportion of necrotic tissue associated with colony development (Ogle and Brown 1971).

The widespread association between invasion in resistant varieties and occurrence of necrotic tissue in the host has led to the concept of a causal relationship between hypersensitivity and resistance (Hooker, 1967). This causal relationship has never been seriously challenged, although resistance is not always associated with cell collapse (Brown et al. 1966). However, Heath (1971, 1976, 1980) has suggested that hypersensitive response play a causal role in disease resistance. In this paper, therefore, infection development of the disease, its host range and the mechanisms involved in the resistance responses of different host and non-host plant species to the *U. viciae-fabae* is reported.

Materials and Methods

Plant materials

Different legume species faba bean (*Vicia faba* L.), Pea (*Pisum sativum* L.) cvs Greenshaft, Kelvedon Wonder, Feltham First, Waverex, Tufted Vetch (*Vicia cracca* L.), Sweet pea (*Lathyrus odorutus* L.) cvs. Beaujolais, Air Warden, French bean (*Phaseolus vulgaris* L), Cowpea (*Vigna unguiculata* L.), Soybean (*Glycine max* L.) cvs

Magoye, Hernon, White clover (*Trifilium repens* L.), and Hop trefoil (*Trifolium campestre* L) including known hosts and non-hosts of *U. viciae-fabae* were grown in the glasshouse in polythene pots of 10 cm diameter in soil test compost under normal agronomic practices at Wye College during 1998. In each pot two plants were grown and kept in the glasshouse until they were ready for inoculation. Tufted vetch, white clover and Hop trefoil were included in the study from a field where they had grown naturally in Wye area.

Inoculation procedure

Frozen urediospores of *U. viciae fabae* collected from a field at Wye College (United Kingdom) were mixed with talc in the ratio of 1:20. Before inoculating the test plants spore viability was tested by inoculating them to faba bean (a susceptible host). After confirmation that the spores were viable the mixture was applied uniformly to the lower leaf surface of each variety. Approximately 2-4 mg of spores was used depending on the size of the leaf with the help of fine paint brush on pre-moistened plants. The inoculated plants were immediately covered with a moistened polythene bag for the next 48 hours to maintain high humidity in order to facilitate spore germination and were kept on a laboratory bench. Thereafter, the plants were transferred to a growth room and kept at 20-22 ^{0}C and illuminated for 16 h/day at 12820 lx throughout the experimental period. For each plant species two plants were inoculated. Plants such as Cowpea and French bean and Soybean the cotyledon leaves and the first true leaves were inoculated, respectively. Pea and sweet pea cultivars were inoculated at fully expanded two bifoliate leaf stages. Tufted vetch, white clover and Hop trefoil the youngest fully expanded leaves were inoculated at any stage found in the field (Ghmire 1995).

Tissue clearing procedure

Inoculated leaf samples were collected from each plant 5 and 12 days after inoculation and cut into small pieces of about 1 cm^2 and immersed in 95% methanol overnight. Thereafter half of the tissues were placed in chloral hydrate alone and half of them with added 0.25% Trypan blue for 4-6 hours to stain the tissue. The tissue was mounted in 50% glycerol for the study of the behaviour of the fungus on and in the plant tissue and host response under Nomarski optics, light microscope and Epifluorescence (Crucefix and Mansfield 1983).

Microscopy

Behaviours of the fungus on and in the plant tissue, intercellular fungal growth and host and non-host responses to the pathogen were observed with the help of Zeiss Axioplan 2 microscope fitted with a MINOLTA R. D. 175 Digital Camera. Observations were made with Nomarski Differential Interference Contrast (DIC) method under different magnifications. Behaviour of the fungus and host responses was recorded by taking pictures with the Digital Camera under different Nomarski optics and UV excitation conditions. Quantitative data were also taken from 50 spores to record germination, stomatal location, penetration, haustorium formation at each infection site and host responses. Chi square (X^2) test were used to analyze the data using Gene Stat Package.

Results

The response of the test plant species to pustule production of *U. viciae-fabae* is presented in Table 1. Faba bean and pea cultivar Kelvedon Wonder showed very dense sporulation. Feltham First and Waverex showed densely and medium dense sporulation, respectively. Greenshaft showed extremely very few numbers of sporulation. The pustules produced on this cv. were delayed by 3 days as compared to faba bean and other susceptible cvs of pea. All the other test plants revealed no sporulation.

Table 1. The sporulation of *Uromyces viciae fabae* on different plant species.

Plant species	Reaction
Faba bean	Very dense sporulation
Pea cv. Greenshaft	Very few sporulation
Pea cv. Kevedon Wonder	Very dense sporulation
Pea cv. Feltham First	Dense
Pea cv. Waverex	Medium dense
Sweet Pea cv. Beaujolais	None
Sweet Pea cv. Air Warden	None
Tufted vetch	None
French bean	None
Soybean cv. Hernon	None
Soybean cv. Magoye	None
Cowpea	None
White clover	None
Hop trefoil	None

The behaviours of *U. viciae-fabae* on different legume hosts and the responses of these plant species to the pathogen are shown in Tables 2 and 3. The plant species such as faba bean and pea cultivars Kelvedon Wonder, Feltham First, and Waverex which allowed dense sporulation were considered susceptible hosts. Where as the plant species such as Tufted vetch and sweet pea cvs Beaujolais, Air Warden and pea cultivar Greenshaft showed resistant and partial resistant responses and were considered as resistant and moderately resistant hosts respectively. The rest of plants tested for the pathogen were considered to be non-hosts.

Table 2. Behaviour of *Uromyces viciae fabae* on test plants and responses of these plants to the fungus.

Plant species	Spores germinated		Stomata located		Stomata penetrated		No. of haustorium per infection site		Host response	
	5 DAI	12 DAI	5 DAI	12 DAI	5 DAI	12 DAI	5 DAI	12 DAI	5 DAI	12 DAI
Faba bean	36	35	20	21	18	21	7	uncountable	S	S
Pea cv. Greenshaft	35	39	8	6	7	6	3	4	PR	PR
Pea cv. Kevedon Wonder	40	41	15	26	14	24	8	uncountable	S	S
Pea cv. Feltham First	39	33	17	24	13	21	5	uncountable	S	S
Pea cv. Waverex	37	34	19	22	16	20	6	uncountable	S	S
Sweet Pea cv. Beaujolais	37	44	8	5	3	2	3	2	HR	HR
Sweet Pea cv Air Warden	30	38	9	4	2	3	2	2	HR	HR
Tufted Vetch	33	46	29	42	21	34	0	0	HR	HR
French bean	22	19	15	15	6	8	0	0	HR	HR
Soybean cv. Hernon	42	39	9	10	2	4	0	0	HR	HR
Soybean cv. Magoye	35	38	11	12	3	7	0	0	HR	HR
Cowpea	39	42	37	35	25	27	0	0	HR	HR
White clover	33	37	19	28	11	11	0	1	HR	HR
Hop trefoil	32	43	21	41	0	2	0	0	HR	HR
Chi-square (df=13) probability	31.05 0.1	55.39 <0.01	81.35 <0.01	175.25 <0.01	102.3 <0.01	148.41 <0.01				

HR= Hypersensitive Reaction; S= Susceptible; PR= Partial Resistance; DAI= Days after inoculation

Table 3. Comparison between broad bean and other test plants for surface behavior of *Uromyces viciae fabae*.

Plant species	Germinated out of 50				Stomata located out of 50				Stomata penetrated out of 50			
	5 DAI		12 DAI		5DAI		12 DAI		5 DAI		12 DAI	
	)2	p	)2	p	)2	p	)2	p	)2	p	)2	p
FB vs Pea cv. Greenshaft	0.049	0.826	0.832	0.362	**7.143**	**0.008**	**11.416**	**0.001**	**6.453**	**0.011**	**11.416**	**0.001**
FB vs cv. Kevedon Wonder	0.877	0.349	1.974	0.160	1.099	0.295	1.004	0.317	0.735	0.391	0.364	0.547
FB vs cv. Feltham First	0.480	0.489	0.184	0.668	0.386	0.534	0.364	0.547	1.169	0.280	0.000	1.000
FB vs cv. Waverex	2.954	0.822	0.047	0.829	0.042	0.838	0.041	0.840	0.178	0.673	0.041	0.839
FB vs cv. SP Beaujolais	0.051	0.822	**4.882**	**0.027**	**7.143**	**0.008**	**13.306**	**<0.001**	**13.562**	**0.001**	**20.384**	**<0.001**
FB vs cv SP Air Warden	1.604	0.206	0.457	0.499	**5.877**	**0.016**	**15.413**	**<0.001**	**16.000**	**0.001**	**17.763**	**<0.001**
FB vs Tufted Vetch	0.421	0.517	**7.862**	**0.005**	3.241	0.072	**18.919**	**0.001**	0.378	0.539	**6.828**	**0.009**
FB vs French bean	**8.046**	**0.005**	**10.306**	**0.001**	1.099	0.295	1.562	0.212	**7.895**	**0.005**	**8.208**	**0.004**
FB vs Soybea cv. Hernon	2.098	0.148	0.832	0.362	**5.877**	**0.016**	**5.657**	**0.018**	**16.000**	**0.001**	**15.413**	**<0.001**
FB vs Soybea cv. Magoye	0.049	0.826	0.457	0.499	**3.787**	**0.052**	**3.664**	**0.056**	**13.562**	**0.001**	**9.722**	**0.002**
FB vs Cowpea	0.480	0.489	2.767	0.097	**11.791**	**0.001**	**7.955**	**0.005**	1.999	0.158	1.442	0.230
FB vs White clover	0.421	0.517	0.198	0.656	0.042	0.838	1.961	0.162	**4.596**	**0.032**	**4.596**	**0.032**
FB vs Hop trefoil	0.735	.0395	**3.730**	**0.054**	0.041	0.839	**16.978**	**<0.001**	**21.951**	**0.001**	**20.384**	**<0.001**

FB= Faba bean; SP= Sweet pea; P = probability value; Bold = Significant at that level of p value

Behaviour of *U. viciae-fabae* on host plants

Faba bean

According to the results obtained high numbers of the urediospores were germinated and better attachment was observed. Frequent location of stomata and penetration was also recorded. The average spore germination was 70% and out of these 40% located stomata and 36% successfully penetrated through the stomata and produced an average number of 7 haustoria per infection site at 5 days after inoculation (DAI) (Table 2). Extensive intercellular mycelial growth and uncountable numbers of haustoria were produced at 12 DAI. The intercellular mycelial network was extremely complex so that almost all the leaf tissues were invaded with intercellular hyphae and it was not possible to distinguish which hypha belongs to which infection site. In close examination of haustoria, ensheathment of some haustoria was observed (Plate 1). Most of the successfully penetrated sites developed sporulation 10-12 DAI. This type of interaction clearly revealed a susceptible reaction of the host to the pathogen.

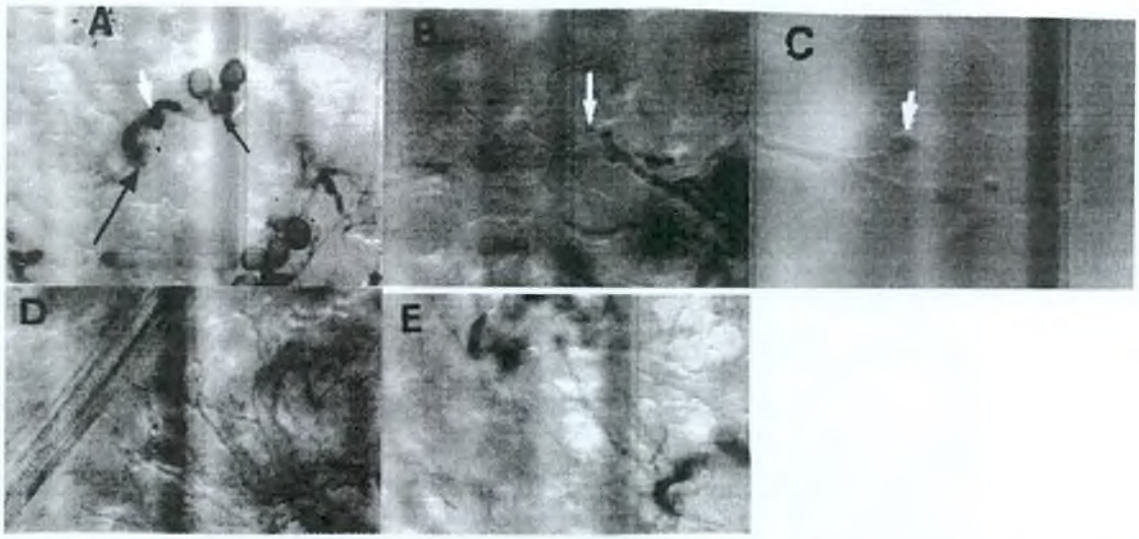

Plate 1. Susceptible reaction of faba bean to *U. viciae-fabae*. (A) germ-tube (white arrow) locating stomata and appressorium formation (long black arrow), spores (small black arrow), (B) ensheathed haustorium, (C) haustorium, (D) intercellular hyphae from a single infection site, and (E) complex intercellular mycelial network from different infection sites.

Pea

Cvs Kelvedon Wonder (KW), Waverex (W) and Feltham First (FF) showed similar response to that of faba bean with better spore germination and almost similar stomatal location, penetration and extensive intercellular hyphal growth. An average number of 8, 6, and 5 haustorium per infection site was recorded for KW, W, and FF, respectively at 5 DAI. All cultivars produced uncountable number of haustoria at 12 DAI (Table 2). Variation in the intercellular fungal growth was also observed among the three pea cultivars as well. Despite these variations the infected tissue of the three cvs showed susceptible reactions as faba bean. On the other hand pea cv. Greenshaft showed poor spore attachment, frequent failure to locate and penetrate stomata (Table 2), slow intercellular hyphal growth, rare sporulation, longer latent period and relatively few numbers of haustoria, compared to faba bean and other pea cultivars. Comparison of the sporulation observed on Kelvedon Wonder and Greenshaft is shown (Plate 3). Some of the infection sites showed rapid collapse of guard cells as well as mesophyll cells with the accumulation of phenolic compounds (Plate 2). This type of reaction revealed that the cultivar has partial resistance to the pathogen. After observation of the variation in susceptibility between pea cultivars, the pathogen was cultured for three consecutive generations on pea cv. KW and reinoculated to faba bean to test whether the initial inoculum was a mixture of races attacking the two hosts. However, the reinoculation of faba bean plants revealed the same reaction as that of the bean rust that was collected from faba bean plants confirming that the same isolate can infect both hosts.

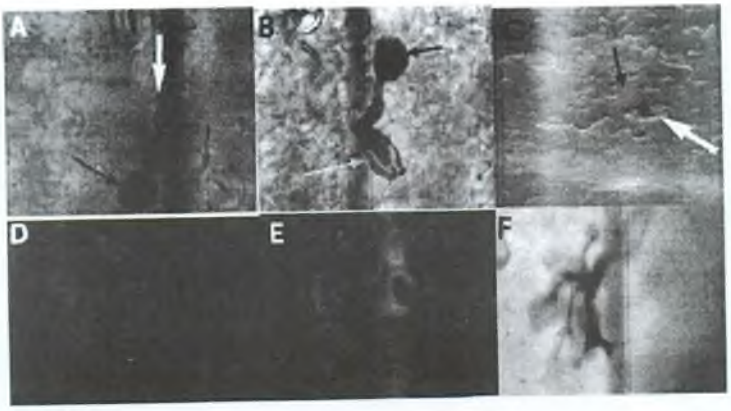

Plate 2. Resistant reaction of pea cv. Greenshaft to *U. viciae-fabae*. (A) failure to locate stomata (small black arrow) by germinated spore (large black arrow) and germ-tube (white arrow), (B) germ-tube produced from a spore (black arrow), locating stomata and appressorium formation (white arrow), (C) guard cell (white arrow) and epidermal cell (black arrow) collapse, (D) mesophyll cell collapse, (E) mesophyll cell collapse violet light, (F) death of mesophyll cell and infection hyphae.

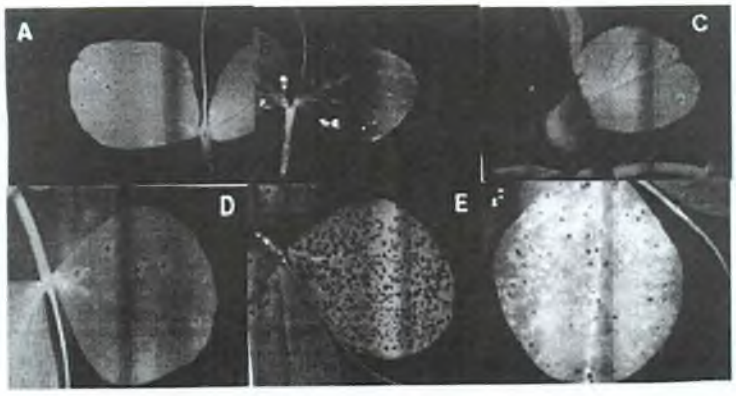

Plate 3. Comparison of *U. viciae-fabae* pustule production on the leaves of partially resistant- and susceptible-cultivars in pea. Partially resistant cv. Greenshaft (A) fungal spread on young lower leaf surface, note the size of pustules, (B) upper leaf surface, (C) very few pustules on the inoculated lower leaf surface. Susceptible cv. Kelvedon Wonder (D) fungal spread on young upper leaf surface, note the size of pustules (E) extremely dense pustules on inoculated lower leaf surface and (F) dense chlorotic and necrotic lesions visible on the inoculated upper leaf surface.

Tufted vetch

Frequent spore germination, better stomatal location and penetration were observed on this plant species with significant difference at 12 DAI in comparison to faba bean (Tables 2 and 3). Death of infection hyphae and massive collapse of mesophyll cells around the infection site occurred immediately after penetration. The extensive dead cells were autofluorescent under different UV excitation filters (Plate 4).

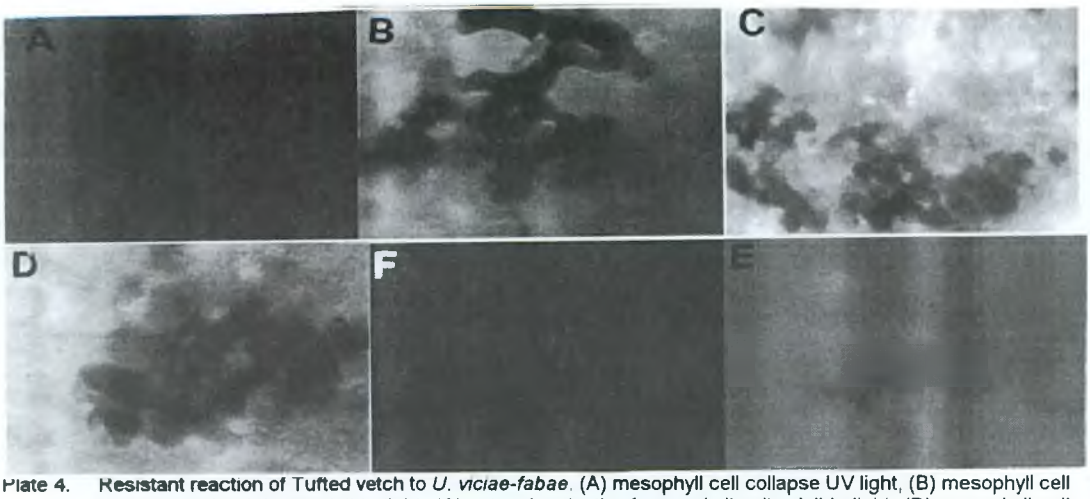

Plate 4. Resistant reaction of Tufted vetch to *U. viciae-fabae*. (A) mesophyll cell collapse UV light, (B) mesophyll cell collapse stained tissue visible light, (C) extensive death of mesophyll cells visible light, (D) mesophyll cell collapse unstained tissue visible light, (E) mesophyll cell collapse violet light, and (F) mesophyll cell collapse blue light.

Sweet pea

Similar spore germination, but an increased failure in stomatal location and penetration on both sweet pea cvs were recorded compared to faba bean. At penetrated sites an average number of two haustoria per infection site was recorded (Table 2). Death of infection hypha and rapid collapse of the haustorium and mesophyll cells were observed between mesophyll cells and in some penetrated plant cells, respectively after deposition of some materials on plant cell and fungal walls similar to tufted vetch. However, the dead guard cells in sweet pea cultivars were autofluorescent but the dead mesophyll cells were not under UV excitation.

Behaviour of *U. viciae fabae* on non-host plants

French bean

Percentages of spore germination and location and penetration of stomata were less in French bean than on faba bean. Except, stomatal location, penetration and germination were statistically significant (Tables 2 and 3). Examination of infection sites showed collapse of both guard cells and mesophyll cells which fluoresced under UV excitation.

Soybean

Poor stomatal location and penetration were recorded in both soybean cultivars with highly significant difference compared to faba bean. Slightly better spore attachment and germination were observed without significant difference (Tables 2 and 3). Infected tissues underwent rapid collapse of the guard cells and mesophyll cells and autofluorescence of phenolic compound (probably lignin) accumulated on cell walls around the collapsed cells were observed under UV excitation.

White clover

Similar spore germination and better stomatal location without significant difference was observed in white clover than faba bean. However, significantly increased failure was registered to penetrate through the stomata when compared to faba bean (Tables 2 and 3). Infected tissue and fungal spore were autofluorescent under UV excitation.

Cowpea

Better spore germination, stomatal location and penetration were recorded on cowpea compared to faba bean (Table 2). However, only stomatal location was statistically significant (Table 3). Close examination of the infected sites showed rapid death of both guard cell and mesophyll cells but in this case the fungus inside the plant tissue fluoresced under UV excitation not the dead cells.

Hop trefoil

Better spore germination and stomatal location were observed compared to faba bean but none of the located germ-tubes were able to penetrate through the stomata particularly at 5 DAI (Table 2). At 12 DAI very few penetrated sites were recorded which resulted in the collapse of mesophyll cells. The dead cells in this plant were not fluoresced under UV excitations but the appressorium above the guard cell was fluorescent.

In summary there were slight differences between the test plant species in spore attachment and spore

germination except for the high and less attachment on soybean cultivars and pea cultivar Greenshaft, respectively. Less spore germination on French bean and frequent spore germination on sweet pea cultivar Beaujolais, Hop trefoil and cowpea were recorded. However, highly significant differences were observed for parameters such as stomatal location and penetration through stomata when the test plants were compared with faba bean using chi-square test (Table 3). Intercellular development of the pathogen showed marked difference between compatible and non compatible host plants.

Discussion

Results on the host species of *U. viciae-fabae*, the faba bean and pea cultivars revealed susceptible reaction except in cv. Greenshaft which showed a slow rusting behavior with a longer latent period. Appearance of pustules on Greenshaft occurred 9 DAI whereas on others susceptible hosts appeared on 6 DAI. In addition to these, very few pustules were produced in Greenshaft. In earlier studies these parameters were used to assess slow rusting behaviour. Bernier and Conner (1982) used these criteria to select slow rusting cultivars of broad bean. Similar criteria were used in wheat and *Puccinia recondita* interaction by Oham and Shaner (1976). The utilisation of the observed slow rusting behaviour coupled with hypersensitive reaction in Greenshaft could be useful characteristics to provide durable resistance.

The development of disease on faba bean after culturing of *U. viciae-fabae* for three generations on pea plants indicates that the same isolate infected both hosts. The significant difference in urediospore germination on leaf surface of the test plants particularly the high germination registered on Hop trefoil and cowpea compared to faba bean suggests the negligible effect of germination inhibitors in these non-host plants while significantly less spore germination in French bean might be due to the possible presence of fungitoxic materials that might interfere with spore germination. However, Heath (1974) and Ghmire (1995) found no difference for spore germination in *U. phaseoli* and *U. viciae-fabae* in host and non-host interaction, respectively.

Poor stomatal location was observed in resistant host cultivars and non-hosts except for hop trefoil and cowpea. Such failure in locating stomata indicates less efficiency of directional growth of the pathogen. The topography of the leaf surface in resistant plants may not be suitable for trophic growth towards stomata. Similar features have bean reported for this pathogen (Ghmire 1995) and cowpea rust (*U. phaseoli* var. *vignae*) on different non-hosts (Heath 1974). The frequent location of stomata on hop trefoil and cowpea might be either the presence of more stomata per unit leaf area and/or absence of physical barriers on the leaf surface towards directional growth.

Frequent differentiation of appressoria away from stomata and formation of infection hyphae on the leaf surface was observed in non-host and resistant host plants. The hypha formed in this way sometimes located stomata but none of these succeeded in penetrating through the stomata. These characters show that the leaf surface on non-host and resistant hosts has some role in resistance.

Distinct differences in ability to penetrate through the stomata were recorded for resistant hosts and non-hosts compared to the susceptible hosts. This indicates early responses of the hosts towards restricting the growth of the fungus which resulted in rapid collapse of guard cells on the resistant hosts and all non-hosts. Clear differences were also recorded in the subsequent intercellular growth of the fungus in the resistant hosts and non-hosts compared to susceptible hosts.

In susceptible hosts the intercellular hyphae progressed without any detectable responses even though some haustorial ensheathment was observed in broad bean. In contrast, the progresses of infection hyphae as restricted in non-hosts and resistant hosts in which detectable resistance responses were recorded at different stages of infection development in different species. The fastest response such as the rapid death of stomatal guard cells, epidermal and mesophyll cells were recorded in all non-host and resistant hosts. The collapse of mesophyll cells suggests that the early collapses of guard as well as epidermal cells were not fully successful to totally restrict the further growth of infection hyphae. It has been described that non-host resistance to *Puccinia graminis* has been restricted by non-host induced substances (Leath and Rowell, 1966). Low levels of such materials at early stage of the infection and increased concentration at later stage of infection might have completely inhibited the fungal growth as suggested by Ghmire (1995).

In most non-hosts and such as tufted vetch no haustorium formation was observed. The prevention of haustorium formation could be due to presence of material deposited in the non-hosts and resistant hosts cell walls, loss of contact between haustorial mother cell with plant cell and death of fungus before haustorium initiation as suggested by Heath (1974).

However, in sweet pea cvs and white clover very small and not well differentiated haustoria were formed. In sweet pea cultivars; death of infection hypha between mesophyll cells and rapid death of haustorium and mesophyll cells in some successfully penetrated sites were observed after deposition of some materials on the cell wall and walls of the fungus. The same observation has been reported on lentil infected with *U. viciae-fabae* (Ghmire 1995) and infections of *U. Phaseoli* var *vignae* to immune varieties of cowpea (Heath and Heath 1971).

Restricted intercellular growth of the fungus was a common feature observed in all non-hosts and resistant hosts cvs. This restriction of the intercellular growth of hyphae could have been due to at least two different mechanisms; (i) HR of the stomatal guard cells, epidermal cells, mesophyll cells at attempted penetration site (ii) the deposition of autofluorescing and non fluorescing substances on the cell walls and walls of infection hyphae.

In this study sweet pea cvs and tufted vetch showed resistant responses to *U. viciae-fabae* although these species are assumed to be hosts of this pathogen in the literature. In earlier studies nine rust races have been identified with some degree of host specialisation. Based on this background it seems logical to consider these two species, resistant hosts rather than non-hosts to this particular isolate.

The plant species tested in this experiment all belong to family Leguminosae, sub family Papilionoidae but belongs to different tribes. Cowpea, French bean, Soybean belongs to tribe *Phaseoleae*; Hop trefoil and white clover to tribe *Trifolieae* (Adams and Pipoly 1980). These two tribes are considered to be non-hosts to *U. viciae-fabae*. On the other hand, the faba bean, pea, sweet pea, and tufted vetch which belongs to tribe *Vicieae* (Kupicha 1974) are all considered host plants to *U. viciae-fabae*. However, different responses to the pathogen were observed within the same tribe and even genus ranging from complete susceptibility in faba bean and some cultivars of pea and partial resistance in pea cultivar Greenshaft and complete resistance in tufted vetch and sweet pea. Such a variation in the same tribe indicates that this particular isolate of *U. viciae-fabae* is more host specific than the other races reported earlier, a conclusion also reached by Ghmire (1995)

All the tested species showed immune response to the pathogen except faba bean and three cultivars of pea that showed susceptible reaction and Greenshaft partial resistance with slow rusting behaviour and HR. This suggests that the isolate has very limited host range. This in turn suggests that this isolate might be economically less important than the other isolates which are reported to have a wider host ranges in literature.

The hypersensitive response of guard cells, epidermal cells, mesophyll cells and deposition of some compounds on cell wall around infection sites and walls of hyphae would limit further fungal growth. For this limited growth, post infectionally induced physical (deposition of phenolic compounds) and chemical barriers (phytoalexins) might have significant roles in the expression of resistant responses. This implies that the observed resistant mechanisms could help to develop varieties with durable resistance to faba bean rust with the help of modern molecular biology techniques.

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