

INTERACTION OF UROMYCES FABAE WITH LEAF SURFACE MICRO-ORGANISMS OF BROAD BEAN IN VITRO AND IN VIVO

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Abstract

The predominant phylloplane micro-organism of broad bean (*Vicia faba* L.) were *Pseudomonas* bacteria *Cryptococcus* and *Sporobolomyces* yeasts and to a lesser extent *Aureobasidium pullulans*. The effect of these micro-organisms on germination of *Uromyces fabae* uredospore on glass and on leaf surfaces and its infection on detached leaves is discussed. Inhibition of germination of *U. fabae* has been associated to the production of antibiotics by *Pseudomonas* bacteria. Stimulation of germination and lesion formation by *U. fabae* in the presence of *Cryptococcus* and *Sporobolomyces* yeasts has been related to the release of stimulants of nutrients of unknown nature.

Introduction

The aerial surfaces of higher plants growing under natural conditions are an important habitat for growth of micro-organisms such as bacteria, yeasts and fungi (Sharma & Garge, 1979; Singh & Rai, 1980; Blakeman, 1981). The growth of the micro-organisms on the leaf surfaces may be beneficial or harmful in relation to defence of host plant against disease. Micro-organisms such as bacteria and fungi inhibit spore germination and infection of all categories of pathogens by number of mechanisms. Blakeman & Brodie (1976) have described basic mechanisms of antagonism which include; direct parasitism, the production of extracellular antibiotics and other substances, nutrient competition on the host and stimulation of host defence.

The present report describes the uredospore germination and germ tube growth of broad bean rust fungus, *Uromyces fabae* in the presence of *Pseudomonas* bacteria, *Cryptococcus* and *Sporobolomyces* yeasts on glass and leaf surface of broad bean.

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Material and Methods

Isolates of *Pseudomonas* bacteria, *Cryptococcus* sp. and *Sporobolomyces* sp. yeasts obtained from leaf surfaces of broad bean were grown separately for two days in shake culture flasks (100 ml), each containing 25 ml. of 1/10 strength of nutrient broth medium. Cells were removed from flasks and washed three times with (10 ml) aliquots of sterilized deionized water on a sterilized filter membrane (pore size $0.45\mu\text{m}$). Suspension of different isolates of *Pseudomonas* bacteria were prepared by resuspending the filter membrane into a universal bottle containing sterilized water and shaking well to get homogenous suspension of the cells. The number of *Pseudomonas* bacterial cells in the original suspension was diluted by adding drops to sterilized deionized water (9 ml) until disappeared faintly turbid. The number of *Cryptococcus* sp., *Sporobolomyces* sp. yeasts cells in the suspension were determined with haemocytometer and then adjusted to give 100,000 cells per ml.

Droplets (20 μl) containing *U. fabae* uredospores $2 \times 10^4 \text{ml}^{-1}$ and *Pseudomonas* bacteria or *Cryptococcus* sp., *Sporobolomyces* sp. yeasts were placed on glass and upper leaf surfaces of broad bean as incubated at 20°C . After 24th incubation leaves and glass slides were removed to assess germination of uredospores based on at least 200 spores in randomly selected microscopic fields. At least 50 germ tubes length were measured by using micrometer eye piece. Assessment of effects of *Pseudomonas* bacteria, *Cryptococcus* sp., *Sporobolomyces* sp. yeasts on *U. fabae* uredospore germination and germ tube growth were calculated using an arcsine transformation to give 95% confidence limits (Sokal & Rohlf, 1969; Blakeman, 1975). A 'T' test was applied to data for measurements of germ tube length. Broad bean detached leaves from plants and spread with the same concentration of isolates of *Pseudomonas* bacteria or *Cryptococcus* sp., *Sporobolomyces* sp. yeasts and uredospores were placed in Petri dishes lined with moist filter paper and incubated at 29°C . At least 10 replicates were used in each treatment and a count of rust lesions on leaf surfaces was made before the epidermis ruptured at the site of each infection. The area of each leaf was measured in cm^2 using leaf measuring meter.

Results

Effect of leaf surface micro-organisms on germination and germ tube growth of *U. fabae* uredospore *in vitro*.

Bacterial and spores of *Aureobasidium pullulans* in simultaneous inoculation reduced both germination and germ tube growth of *U. fabae*

with significant reduction shown by isolate A 1, whereas isolates A 2 & A 3 had much less effect when compared with control spores germinated in water alone (Table. 1) Both *Sporobolomyces* sp. and *Cryptococcus* sp. yeasts enhanced both germination and germ tube growth, especially in the presence of *Cryptococcus* sp. (Table. 1).

The germination of *U. fabae* uredospores was also significantly reduced when a mixture of different isolates of bacteria were applied simultaneously with uredospores on glass surface, but growth of germ tube were slightly reduced (not significant) when compared with control spores germinated alone in water (Table. 1).

Effect of leaf surface micro-organisms on germination, germ tube growth and on infection development of *U. fabae* *in vivo*.

In the leaf surface both germination and germ tube growth of uredospores significantly reduced in the presence of isolate A 1 as compared to isolates A 2 & A 3 (Table. 2) Compared with control spores germinated in water, the length of germ tubes of *U. fabae* were significantly reduced in the presence of isolates A 1 & A 3, whereas isolate A 2 had much less effect on growth of germ tubes (Table-2). Significant inhibition of both germination and germ tube growth of *U. fabae* was observed in simultaneous inoculation of a mixture of different isolates of bacteria with uredospores on a leaf surface (Table. 2).

Infection of detached leaves by *U. fabae* was considerably reduced when inoculated with uredospores and isolates A 1 & 3, but isolate A 2 showed little reduction of rust lesion development (Fig. 1). Simultaneous inoculation of uredospores with spores of *Aureobasidium pullulans* slightly retarded (but not significantly) lesion formation by *U. fabae* on leaf surfaces when compared with water control (Fig. 1). Lesion formation by *U. fabae* on detached leaves was slightly enhanced when uredospores were placed simultaneously with *Cryptococcus* sp. and *Sporobolomyces* sp. yeasts (but not significant) when compared with water control (Fig. 1).

Pre-inoculation of detached leaves with *Cryptococcus* sp. and *Sporobolomyces* sp. yeasts or water for 2-days before inoculation with *U. fabae* uredospores resulted an increase in numbers of lesions formed by rust in the presence of yeasts, whereas presence of water caused a slight reduction in lesion formation (but not significant) when compared with water control (Fig. 2, Plate, 1).

Discussion

Of the isolates tested *in vitro* isolate A 1 caused a marked inhibition

of uredospore germination and germ tube growth of *U. fabae* as compared to isolates A 2 & A 3. In *vivo* however, isolate A 3, which was ineffective *in vitro* showed a considerable effect in reducing germ tube length *in vivo*, whereas isolates A 1 & A 2, showed similar response as found *in vitro*. Differences in antagonistic behaviour of saprophytic micro-organisms under *in vitro* and *in vivo*, conditions have been reported (Fokkema, 1973 & Dickinson and O'Donnel, 1977). This could as well be due to biological variation in the inoculum droplets as described by Blakeman (1972), Spurr (1981). An other possibility which may be related to the variation can be the influence of the host plant on physical nature of the infection drops as suggested by Topps and Wain (1957), Blakeman (1973). Pathogens which are nutrient dependent such as *Botrytis cinerea* for its successful growth on plant surfaces could be severely affected by competition from other saprophytic micro-organisms (Blakeman, 1975; Brodie and Blakeman, 1976).

However, it seems unlikely that biotrophic pathogens such as *U. fabae* are affected by nutrient competition. McBride (1969) has shown that the rust *Melampsora medusae* can be antagonized by bacteria, and concluded that in this interaction antibiotic seemed to be involved but not nutrient competition. It is therefore, apparent from the above discussion that, inhibition of rust uredospore germination on an inert surface and on leaf surface is unlikely to be because of competition, but could be due to production of antifungal or toxic substance(s) by saprophytes.

The germination and germ tube growth of *U. fabae* were inhibited by *Aureobasidium pullulans* *in vitro* and this may be due to the production of an antifungal substance(s) by *A. pullulans* (Baigent and Ogauva, 1960). The presence of *Sporobolomyces* sp. and *Cryptococcus* sp. yeasts with *U. fabae* resulted in stimulation of uredospore germination and germ tube growth *in vitro* and this enhancement may be the result of leakage of nutrients or stimulants from yeasts (Parker, 1982).

Presence of different isolates of bacteria (*Pseudomonas*) with *U. fabae* on broad bean leaves suppressed the pustule development on leaf surfaces, but only one isolate A 2, tested showed no effect on rust development. The similar mechanism of inhibition by bacteria may be involved as discussed in germination and germ tube growth study. Pustule development by rust on surfaces of detached leaves was slightly affected by the presence of *A. pullulans*, and this may have occurred due to the presence of antifungal substance(s) on the leaf surfaces (mansfield and Deverall, 1974). Lesion development of detached leaves by *U. fabae* was remarkably enhanced in the presence of yeasts. An increase in lesion formation by rust may be related to the release of nutrients or other substance(s) of unknown

nature (Parker and Blakeman, 1983b). It is apparent from the results and discussion that interaction between rust uredospores and phylloplane micro-organisms of broad bean leaves is very complex. However, it may be important to attempt to understand at least part of these interactions in order to formulate any kind of effective biological control method against the rust, *U. fabae* on broad bean leaf surface.

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Table-1

Effect of simultaneous inoculation with bacteria and yeasts on germination and germ tube growth of *Uromyces fabae* on glass.

Treatment	<i>U. fabae</i> % Germination with confidence limits	Germ tube length(μm)
Control	89 (86-92)	76
*Isolate A 1	71 (66-75)	29 ^a
*Isolate A 2	85 (82-90)	70 n.s.
*Isolate A 3	83 (78-88)	65 n.s.
<i>Aureobasidium pullulans</i>	79 (74-84)	37 ^a
<i>Sporobolomyces</i> sp.	92 (89-96)	124 ^a
<i>Cryptococcus</i> sp.	96 (94-99)	346b
All isolates together (A 1, A 2 & A 3)	75 (70-82)	64 n.s.

a p = > 0.01 when compared with germinated spores in water.

b p = 0.001 when compared with control germinated spores in water.

n. s. = not significant when compared with control germinated spores in water.

*Probably *Pseudomonas*.

Table-2

Effect of simultaneous inoculation with bacteria on germination and germ tube growth of *U. fabae* on intact leaves

Treatment	<i>U. fabae</i> % Germination with confidence limits	Germ tube length(μ m)
Control	74 (71-81)	103
*Isolate A 1	65 (61-69)	72 ^b
*Isolate A 2	73 (68-78)	91 n.s.
*Isolate A 3	65 (64-76)	76 ^a
All isolates together (A 1, A 2 & A 3)	56 (51-64)	66 ^b

a p = 0.05 when compared with control spores germinated in water.

b p = < 0.01 when compared with control germinated in water.

n. s. = not significant when compared with control spores germinated in water.

*Probably *Pseudomonas*.

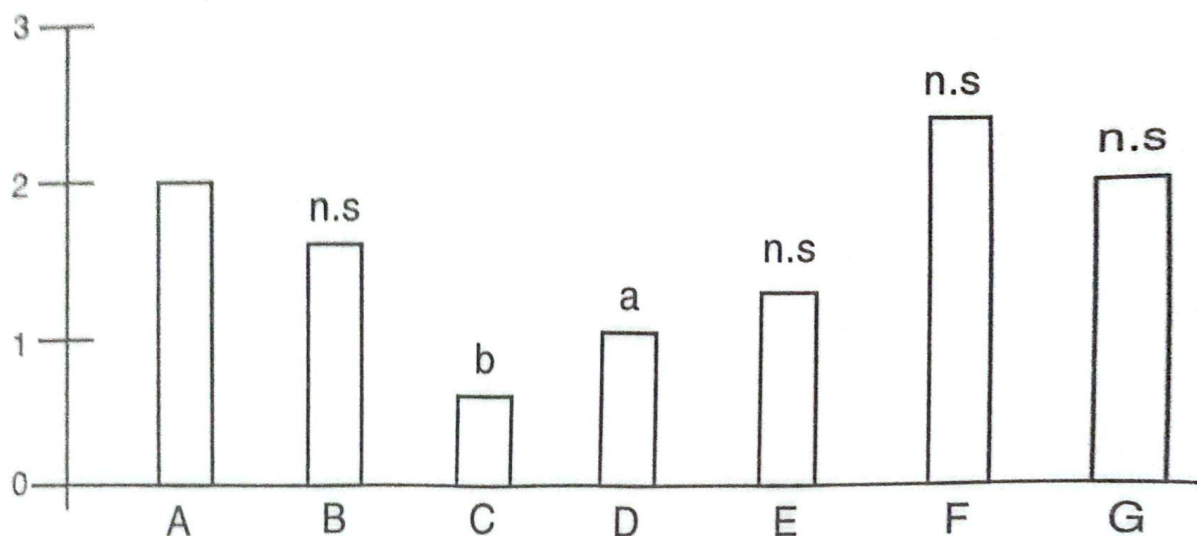


Fig. 1. *Uromyces fabae* lesion development on detached leaves which were simultaneously inoculated with *U. fabae* uredospores and bacteria or *Aureobasidium pullulans* or yeasts.

- A = Control-freshly detached leaves simultaneously inoculated with *U. fabae* uredospores and water.
- B = Leaves simultaneously inoculated with *U. fabae* uredospores and Isolate A 2.
- C = Leaves simultaneously inoculated with *U. fabae* uredospores and Isolate A 3.
- D = Leaves simultaneously inoculated with *U. fabae* uredospores and Isolate A 1.
- E = Leaves simultaneously inoculated with *U. fabae* uredospores and *Aureobasidium pullulans*.
- F = Leaves simultaneously inoculated with *U. fabae* uredospores and *Cryptococcus* sp.
- G = Leaves simultaneously inoculated with *U. fabae* uredospores and *Sporobolomyces* sp.

a p = < 0.05 when compared with control.

b p = 0.01 when compared with control.

n.s. = not significant when compared with control.

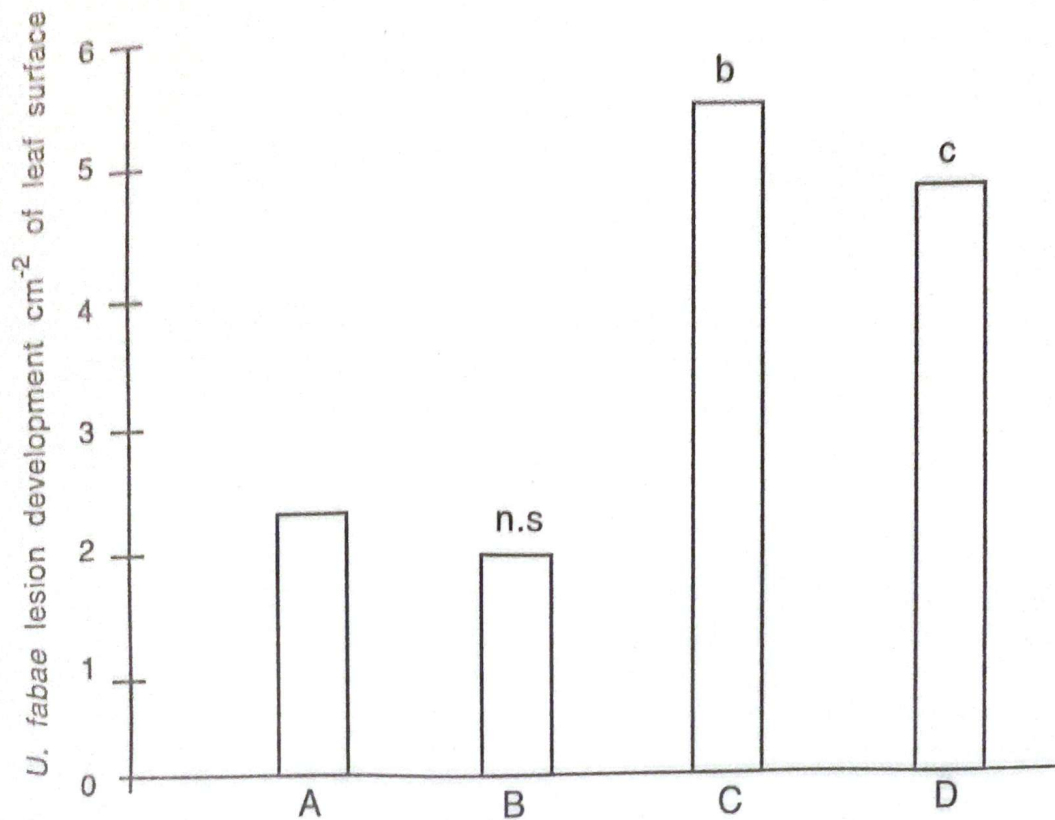


Fig. 2. *Uromyces fabae* lesion development on detached leaves which were sprayed with a suspension of yeasts or water for 2-days before *U. fabae* uredospores inoculated.

- A = Control-freshly detached leaves simultaneously inoculated with *U. fabae* uredospores and water.
- B = Leaves inoculated with water for 2-days before *U. fabae* was inoculated.
- C = Leaves inoculated with *Cryptococcus* sp. for 2-days before *U. fabae* was inoculated.
- D = Leaves inoculated with *Sporobolomyces* sp. for 2-days before *U. fabae* was inoculated.
- a p = < 0.05 when compared with water control.
- b p = < 0.01 when compared with water control.
- n.s. = not significant when compared with water control.

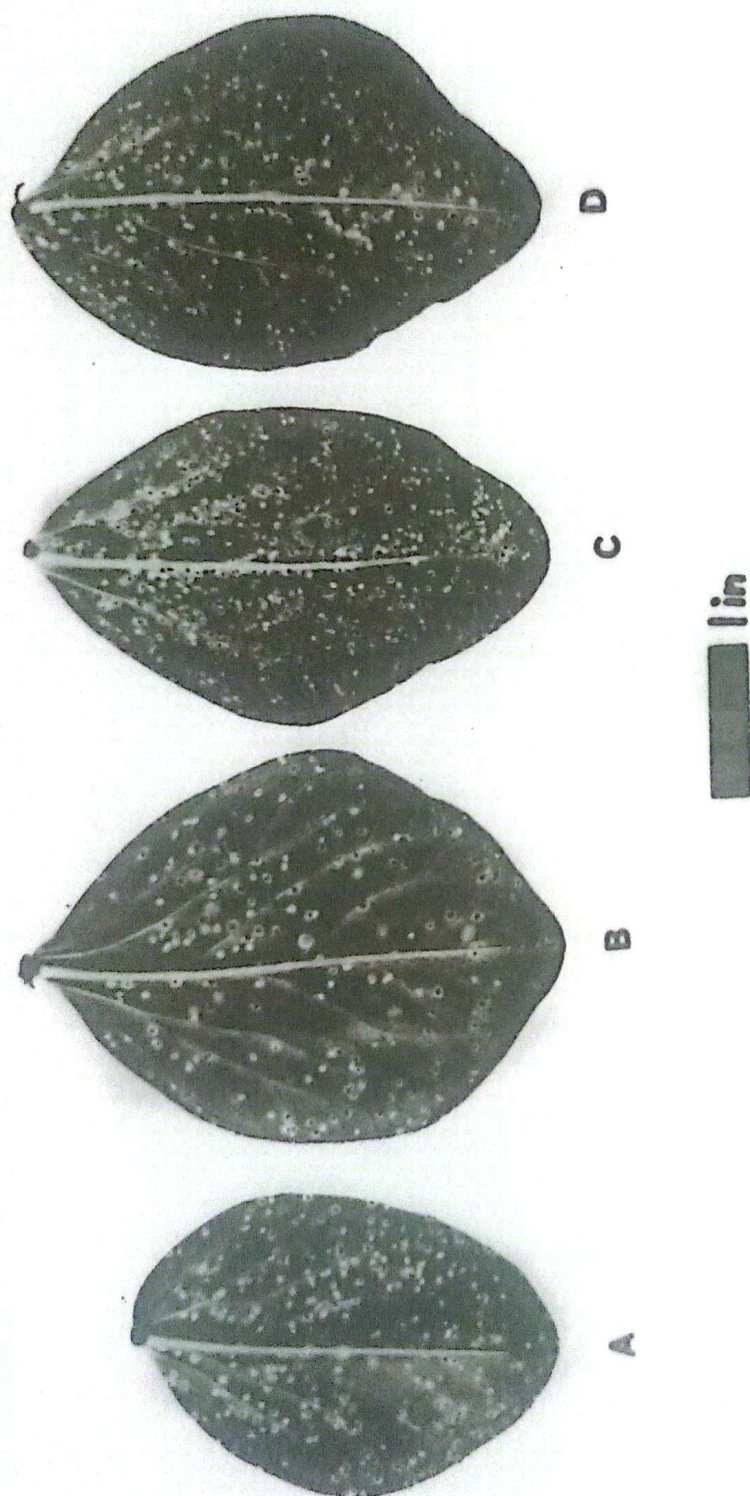


Plate 1. *Uromyces fabae* lesion development on detached leaves which were sprayed with a suspension of yeasts or water for 2-days before *U. fabae* uredospores were inoculated.

A = Control - freshly detached leaves simultaneously inoculated with *U. fabae* and water.

B = Leaves inoculated with water for 2-days before *U. fabae* was inoculated.

C = Leaves inoculated with *Cryptococcus* sp. for 2-days before *U. fabae* was inoculated.

D = Leaves inoculated with *Sporobolomyces* sp. for 2-days before *U. fabae*