

Effects of leaf age of faba bean and inoculum level of *Uromyces viciae-fabae* on pustule development and spore production

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Abstract

Leaf age and inoculum level are important factors for the development of rust disease epidemics in faba bean. The effect of these two factors on spore production and pustule density of rust caused by *Uromyces viciae-fabae* on faba bean was studied in growth room at Wye College in 1998. Three leaf ages and four levels of inoculum density were used in a crossed and nested design. High pustule number was recorded on young leaves followed by middle and then old leaves in all inoculum levels. In terms of overall spore yield, young leaves gave more spore mass than middle aged leaves. Application of highest inoculum level resulted in the highest pustule number. Generally, pustule number significantly ($P < 0.001$, $df=2$) decreased while spore production increased with decreasing inoculum levels ($P < 0.001$, $df=3$). Regression analysis of spore weight (dependent variable) and pustule number (independent variable) showed an inverse relationship ($R^2 = 0.88$ and $R^2 = 0.83$) while spore weight (dependent variable) and pustule size (independent variable) showed positive relationship ($R^2 = 0.89$ and $R^2 = 0.80$) all with significant differences between young and middle aged leaves respectively. In this experiment younger leaves were found to be more susceptible to rust than old leaves. Interaction of leaf age and inoculum level was observed for rust development.

Introduction

Production of spores is of paramount importance for successful parasitism in most fungal-plant interactions that may vary among different pathogens and within host species involved. Such variations can be more pronounced with the developmental stages of the plant or of the plant organ infected (Eskes & Toma-Braghini 1982). Pustule number and size are also affected by these factors. Ghmire (1995) reported that older broad bean leaves were more resistant than younger leaves when infected with *Uromyces viciae-fabae*. The resistance level of oat leaves attacked by *Erysiphe graminis* f. sp. *avenae* was highest when the leaves were just fully expanded, and thereafter susceptibility increased (Jones & Hayes 1971). Cole (1966) found that tobacco leaves were not infected by *Erysiphe cichoracearum* until they were almost fully expanded. In a study on barley seedlings infected by *E. graminis* f. sp. *hordei* (Lin & Edwards 1974) an increase in the

resistance, of both resistant and susceptible hosts, with increasing leaf age was observed.

The idea of inoculum potential has been largely developed for diseases, particularly for soil-borne plant pathogens, though it is applicable to other types of pathogens (Campbell 1989). The aim is to define how much inoculum is needed to initiate disease and how well a pathogen will colonize. He also suggested that if there is some way of measuring inoculum, it may seem obvious that the more the inoculum the severe the infection will be. However, there may be a certain amount of inoculum to cause disease. To overcome the host's defence systems multiple attacks at several different points or several propagules at each point may be needed to establish infection. There is, therefore, a critical inoculum level below which no infection occurs.

The amount of inoculum required to produce infection varies with the pathogen and the host, depending on the relative virulence of the pathogen and the degree of resistance of a particular host. The severity of disease is therefore determined not only by the actual quantity of inoculum, but also by many other factors that have been combined in the concept of inoculum potential (Horsfall & Cowling 1978). Inoculum potential is the sum of all factors that contribute to successful infection of a host by a pathogen and it is one of the main determinants of the amount of disease produced. In this paper, results of a study conducted on spore production, pustule number and size, leaf age and inoculum density are presented and discussed.

Materials and Methods

Faba bean plants (*Vicia faba* L. cv. Aquadulus) were grown in polythene pots filled with compost mixed soil in greenhouse at Wye College during the summer season in 1998. Frozen urediospores of *U. viciae-fabae*, collected from Wye area were used as inoculum and applied approximately equal amounts of the inoculum with the help of fine paint brush on each inoculated leaf at concentrations of 1:10, 1:20, 1:40, and 1:80 spore to talc ratio.

The leaf age treatments were three stages of leaves, viz young, middle and old. The plants were inoculated at the six fully expanded bifoliate leaf stage in a laboratory bench. The second, fourth and sixth bifoliate leaves from bottom to shoot apex wards were considered as old, middle and young leaves respectively. One leaf at each age category was inoculated on two plants with each inoculum level in a crossed and nested design. The inoculated plants were immediately covered with pre-moistened polythene plastic bags sprayed with tap water for 48 hours. These plants were transferred and kept in the growth room throughout the experimental period.

Pustule number on each inoculated leaf was

recorded randomly from four spots with area of 1 cm² on the upper surface of the leaf, two from each plant at an interval of 2 days from 12 days after inoculation (DAI). Total spore production from young and middle aged leaves from each inoculum level were assessed by collecting urediospores that were weighed by a Sartorius 4503 micro sensitive balance with 0.001 mg accuracy four times at an interval of two days to allow spores to develop. The corresponding pustule number per leaf was counted which was used to convert spore production per pustule. Pustule diameter was measured on cleared tissue in methanol over night and 2-4 hours in chloral hydrate in m using eyepiece micrometer fitted into compound microscope at 100x magnification. The relationship between pustule number and size with spore production efficiency was determined using regression analysis.

Results

There were significant differences in pustule production between inoculum levels for each recording date (Fig. 1). The change in pustule number at different recording dates for inoculum levels' 1:10 and 1:20 was relatively low particularly on young leaves. However, the rate of change in pustule number from first to next recording date was slightly higher for inoculum level 1:40 and 1:80. Differences between different leaf ages in pustule production were also highly significant ($p < 0.001$) for each recording date (Fig. 2).

At all inoculum levels, young leaves produced the highest number of pustules followed by middle and old aged leaves (Fig. 2). In all leaf age levels the inoculum density 1:10 gave more pustule number than other levels during the experimental period followed by 1:20, 1:40 and 1:80 inoculum levels (Fig. 3 & 5). However, inoculum level 1:20 was exceeded by the lower inoculum levels, 1:40 and 1:80 in middle aged leaves after 14 DAI (Fig. 4).

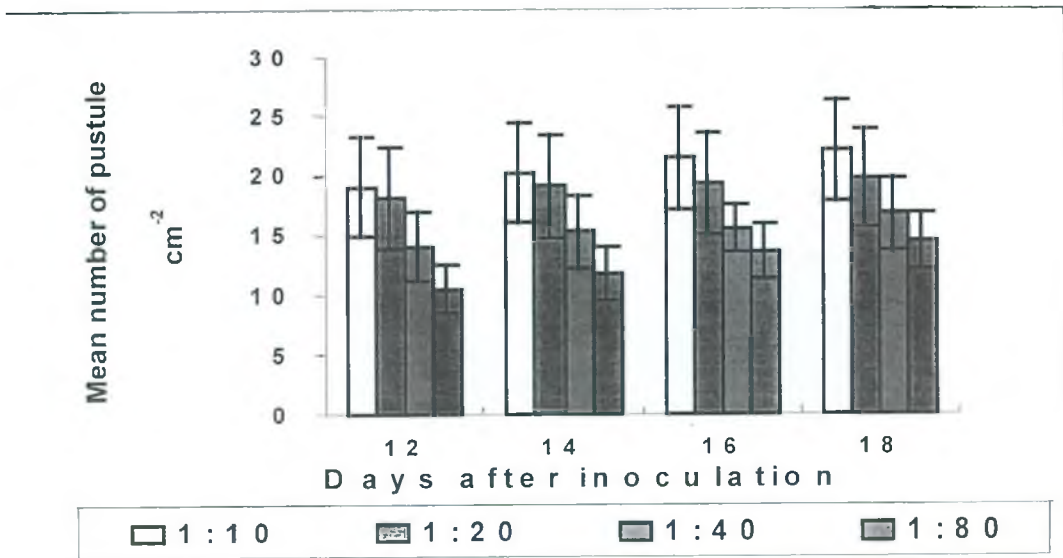


Fig. 1. Effect of inoculum level on pustule production. Bars indicate \pm SEM.

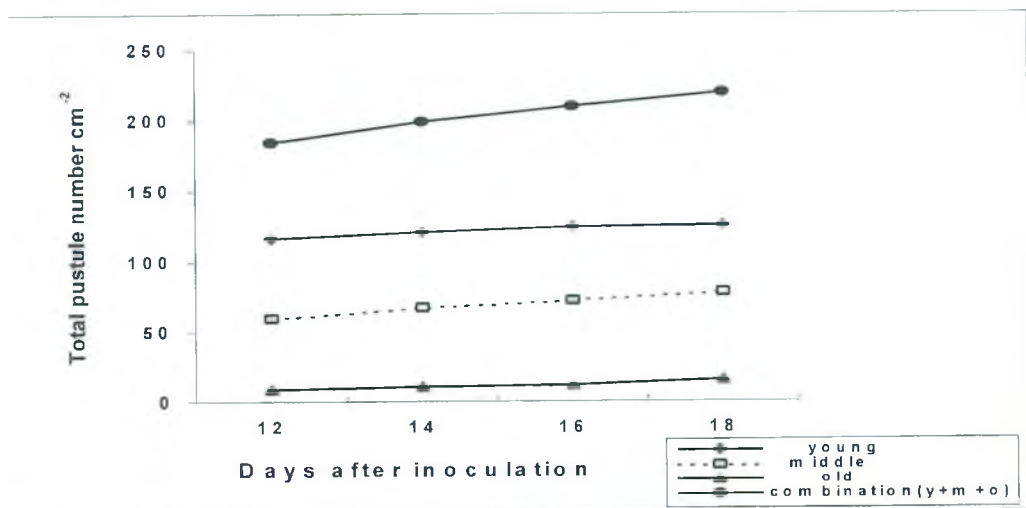


Fig. 2. Total pustules produced at different leaf age at different recording dates.

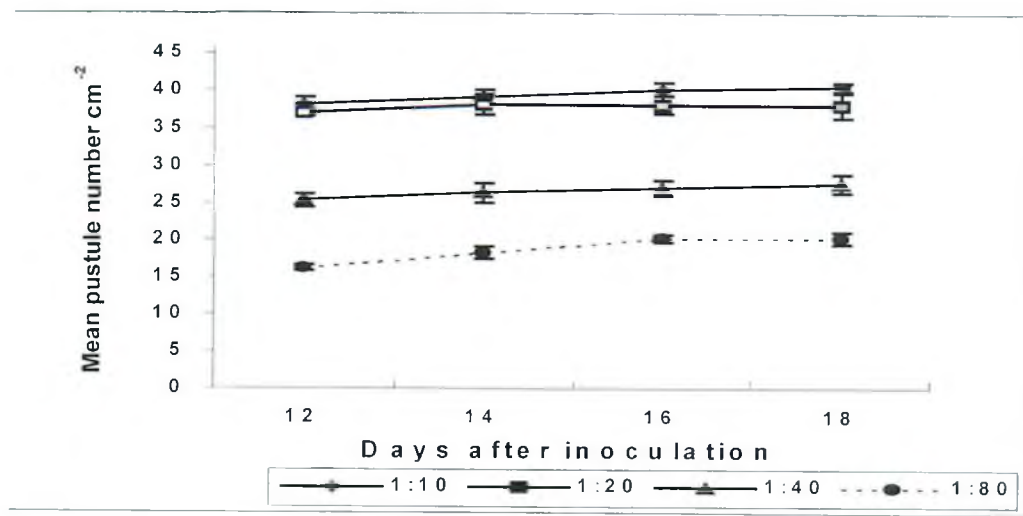


Fig. 3. Effect of inoculum levels on the number of pustules produced on young leaves. Bars indicate SEM.

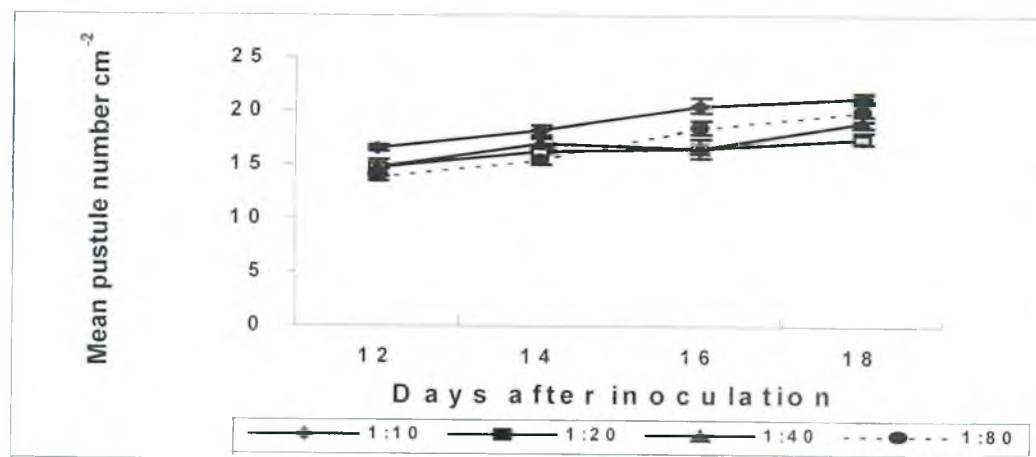


Fig. 4. Effect of inoculum levels on the number of pustules produced on middle aged leaves. Bars indicate SEM.

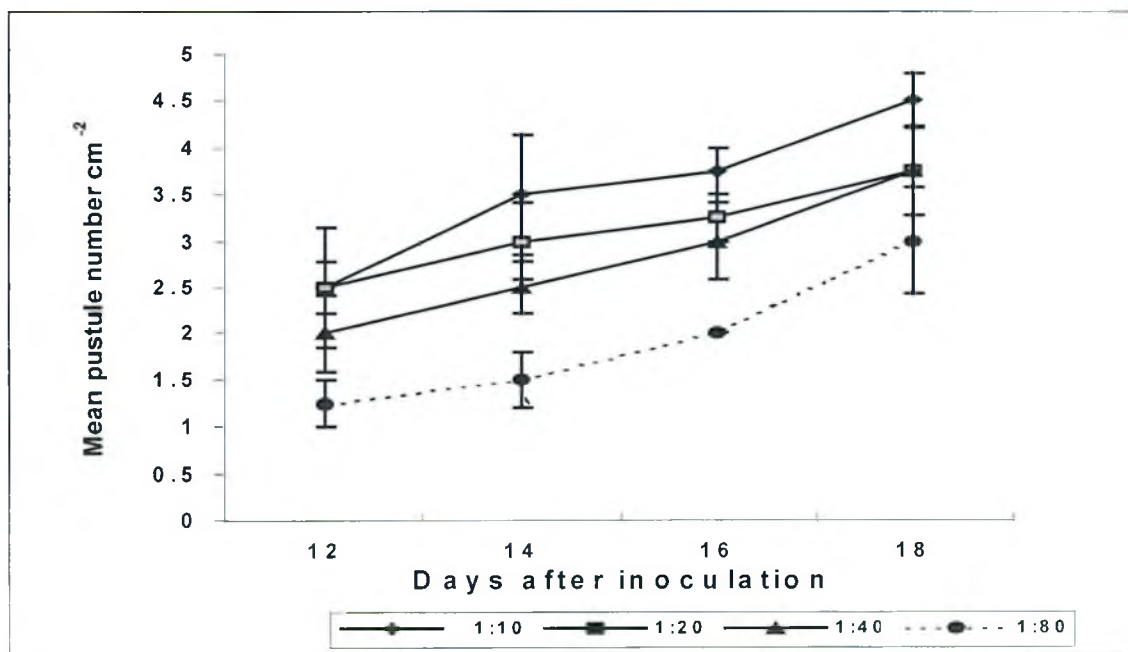


Fig. 5. Effect of inoculum levels on the number of pustules produced on old leaves. Bars are SEM.

The results of spore production efficiency showed that young leaves gave higher spore yield than middle aged leaves (Fig. 6). No measurable amount of spores were obtained on old leaves at all inoculum levels.

The inoculum level that produced the highest pustule number gave less spore weight per pustule and produced small pustule. The spore production efficiency and pustule size increased with decreasing

inoculum level at each leaf age. The regression analysis showed an inverse relationship between pustule number and the spores produced per pustule with highly significant differences both for young ($R^2 = 0.88$) and middle ($R^2 = 0.83$) ages (Fig. 7 a, b). Pustule size and spore weight per pustule showed positive relationship with highly significant difference for young ($R^2 = 0.89$) and middle ($R^2 = 0.80$) leaves (Fig. 8 a, b).

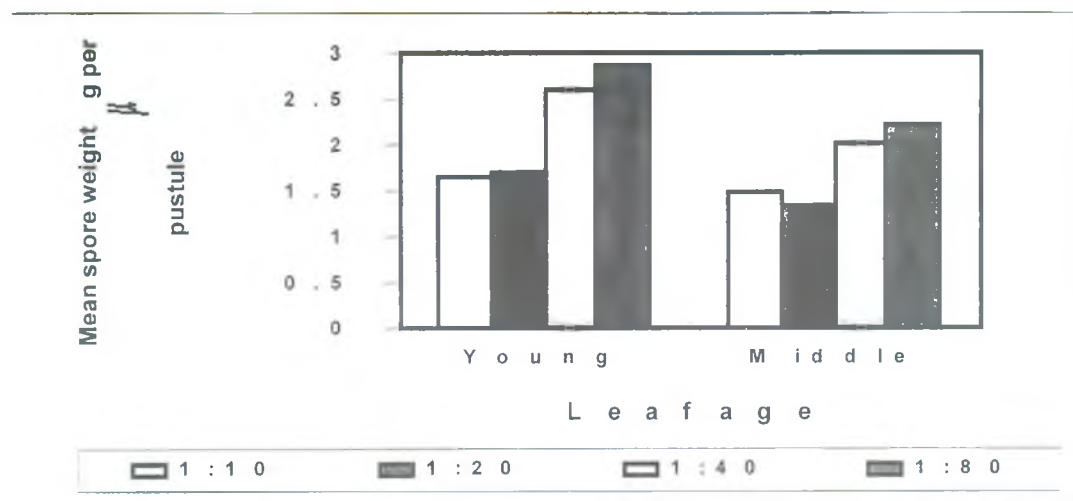


Fig. 6. Spore production efficiency in young and middle leaves inoculated with different inoculum level between 12 to 18 DAI.

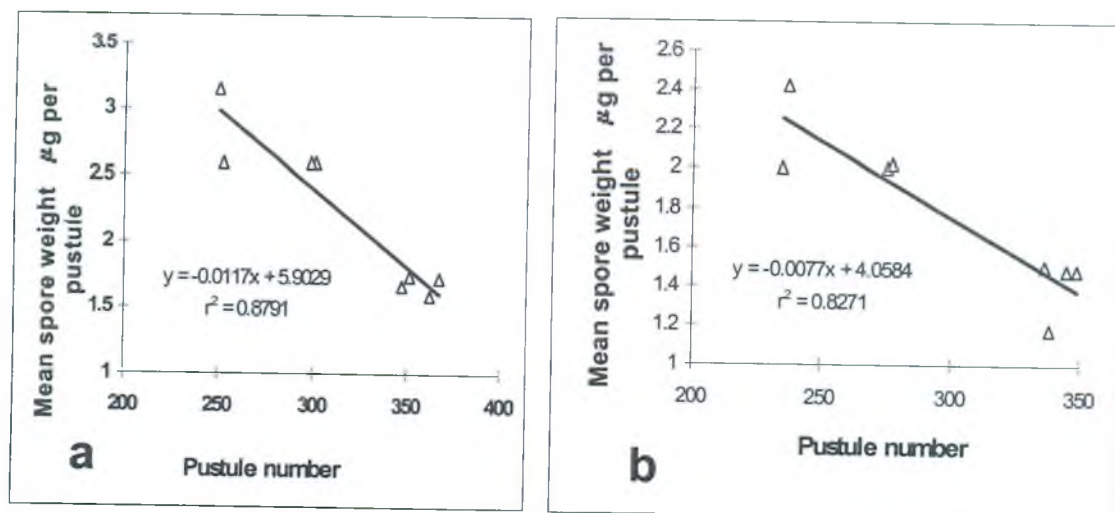


Fig. 7. Relationship between pustule number and spore weight per pustule between 12 to 18 DAI for different inoculum levels (a) young leaves (b) middle aged leaves.

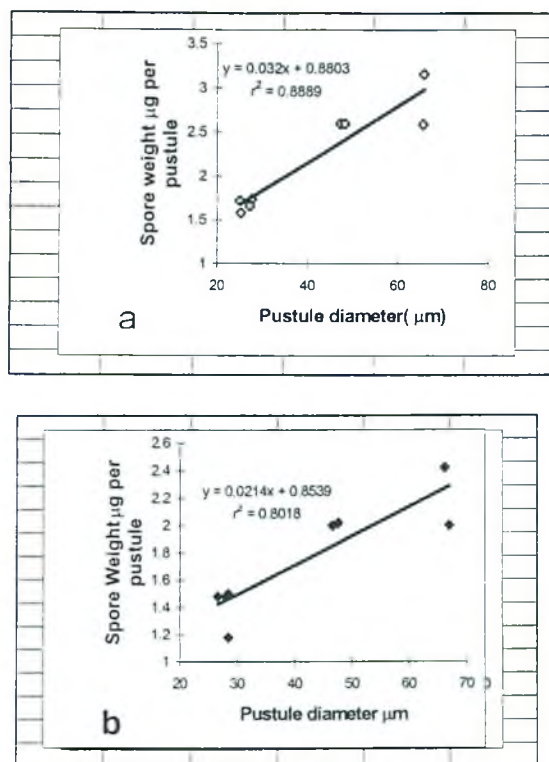


Fig. 8 Relationship between pustule size and spore weight per pustule between 12 to 18 DAI for different inoculum levels (a) young leaves (b) middle aged leaves.

Discussion

Hyde (1977) reported that plants become more resistant when they mature and this may be followed by a decrease with further ageing (Jones & Hayes 1971). However, the effect of age on infection appears to depend on particular host pathogen interaction. Padmanabhan and Ganguly (1954) in Eskes and Toma-braghini (1982) observed that resistance of rice to *Helminthosporium* decreased with age but the resistance to rice blast increased with age. In many cereal rust diseases, an increased resistance to infection was reported to increase with advancing plant age (Hyde & Elahinia, 1989). Eskes and Toma-Braghini (1982) also reported that the effect of leaf age varies with particular coffee species and *Coffea arabica* selections.

an increased resistance with increasing leaf age. A similar effect was reported on susceptible coffee genotypes infected with coffee rust pathogen. Jennings *et al* (1990) found that in the *Allium porrum*-*Puccinia allii* interaction, pustule density, pustule length and colony length decreased with increasing leaf age and observed a tendency for latent period to increase with increasing plant age. But in this study very little or no effect was observed on the latency period of bean rust pathogen in the different age classes. However, significantly distinct variation was recorded in pustule number. A similar result was reported on latent period by Ghmire (1995) for the same fungus and by Jennings *et al.* (1990) for the *A. porrum*-*P. allii* interaction.

This study of *U. viciae-fabae* on broad bean revealed

Differences in pustule production of bean rust fungus

on different aged leaves of broad bean may be due to the differential germination of urediospores. A study conducted on the germination of urediospores of *Hemileia vastatrix* on coffee leaves of different ages revealed that germination was greater on young leaves, compared to intermediate and old leaves (Nutman & Roberts 1963). Russel (1976) reported that germination of *Puccinia striiformis* urediospore on adult *Triticum aestivum* leaves with durable resistance showed higher germination on younger than older leaves.

Secretion of preformed fungitoxic secondary plant metabolite from senescing older leaves to the leaf surface might directly hinder the germination of urediospores. Martins et al. (1986) observed that preformed fungitoxic compounds present in the control leaves disappeared after inoculation. Ghmire (1995) suggested that the presence of toxic material produced by the resident microflora or mycoparasites on leaf surface of older leaves might prevent urediospore germination. Martins, et al. (1986) studied the significance of non-pathogenic fungus induced host metabolites with antifungal activity and reduced germination of *H. vastatrix* urediospores. Such metabolites increased in concentration as leaves aged. The higher number of pustule recorded in this study on this study on the younger leaves followed by middle aged and old leaves in that order. The other possible reason for highly reduced number of pustules on the older leaves could be the poor nutritional relationship between the fungus and the physiologically less active senescing old leaves.

In "compound interest" diseases such as rusts, powdery mildew and downy mildew are polycyclic, resulting in an exponential buildup of the disease in a relatively short period of time. In this case the effect of inoculum level is transient because once the first infection is established the pathogen produces more inoculum on the affected parts. This might lead to the spread of the pathogen and development of the disease on other plants throughout the crop resulting in severe epidemics provided that favorable environmental conditions prevail (Parry 1990)

In this study, an increase in pustule number was found to be directly proportional to the increased level of inoculum. Ghmire (1995) has been reported that an increase in pustule number to be associated with an increased levels of inoculum. He also found

that as the number of pustule increased, their corresponding size was found to be smaller. All inoculum levels resulted in the appearance of pustule as well as sporulation at the same time suggesting the absence of variation in latency period between the different inoculum levels. Synchrony in the appearance of pustule and sporulation in different inoculum level may be due to the time required to colonise available tissue in leaves inoculated with variable inoculum levels. The appearance of secondary pustules on leaves inoculated with low inoculum level may be due to the availability of sufficient nutrients with large leaf area. However, Ghmire (1995) suggested that the appearance of secondary pustules on leaves inoculated with low inoculum level might be due to slower and confined growth of the fungus.

In this study leaves inoculated with high inoculum level developed more small sized pustules compared to those inoculated with low inoculum levels. This might be due to the competition of the intercellular hyphae for limited nutrients per available space in leaves inoculated with high inoculum level. In contrast, fewer numbers of intercellular hyphae to share nutrients available per larger space in the leaves inoculated with low inoculum level has got access to more nutrients for producing large pustules with more spore mass. Results therefore suggest that available space might have significant role on spore production. Accordingly, spore production in this study was found to increase as the inoculum levels were decreased and young leaves were found more susceptible to bean rust than older leaves. This result implies that inoculation of young leaves with bean rust (*U. viciae-fabae*) at an inoculum density of 1:10 spore to talc ratio could be used for screening grain legumes for disease resistance.

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