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EFFECT OF BROAD BEAN LEAF AGE AND INOCULUM LEVELS ON SPORE PRODUCTION EFFICIENCY OF UROMYCES VICIAE-FABAE

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ABSTRACT

Uromyces viciae-fabae **is one of the major production constraints of broad bean (*Vicia faba* L.) and other many tropical legumes, and is world-wide in distribution. The plant age and inoculum level are important host and pathogen factors respectively in the disease epidemiology. Five inoculum levels (1:50, 1:100, 1:500, 1:1000 and 1:2000 w/w) were tested on three, different aged broad bean leaves (young, middle aged and old) for pustule production and spore production efficiencies under controlled environment at Wye College, UK in 1995. The standard inoculum level (1:50 w/w) gave the highest number of pustules and the other inoculum levels were indifferent for pustule production. The highest pustule numbers were recorded from the young leaves followed by middle-aged and old leaves. Total spore production per bifoliate was the highest from young leaves and it was followed by middle aged and old leaves irrespective of the inoculum levels. However, there was an inverse relationship between pustule number and spore production efficiency on young (r^2=0.734; p<0.001) and middle-aged (r^2=0.379; p<0.001) leaves and no such relationship was observed on old aged leaves. This study shows an important role of leaf age and inoculum level in the broad bean rust disease development.**

Additional Key Words: Simple interest, Compound interest, Basidiocarp

INTRODUCTION

Broad bean rust (*Uromyces viciae-fabae*) is the most serious diseases of Faba bean. The disease is worldwide in distribution (Laundon and Waterson, 1965), and is particularly common throughout the Mediterranean region. Pathogen may attack to the crops at any stage of development and young plants are more susceptible than older ones. An early infection is always associated with high yield losses, up to 50% in Northern Delta region of Egypt. Williams (1978) reported that early and severe infection could result as high as 45% yield loss. Normally losses range from 5 to 20% (Ibrahim *et al,* 1978). It infects other crops also, such as pea (*Pisum sativum*), lentil (*Lens culinaris*), vetch (*Vicia sativa*) and many other cultivated and wild species of *Vicia* (Guyot, 1957).

The role of leaf age and amount of initial inoculum are important factors in the establishment of parasitic relationship in many host-pathogen interaction. The leaf age has a major effect on the development of the infection structure on the leaf surface and subsequent sporulation (Countinho *et al*, 1994). In general resistancy increases with increasing plant age (Hyde, 1977) and eventually followed by a decreases in resistance as the plant senesces (Parlevliet, 1975).

A critical minimum inoculum level is required for the development of an epidemic. The role of initial inoculum level is particularly important in the case of "simple interest" diseases such as take all of the cereals (*Gaeumanomyces graminis*), club root of brassicae (*Plasmodiophora brassicae*), panama disease of banana (*Fusarium oxysporum f.sp. cubunse*) than in the case of "compound interest" diseases such as rusts, powdery mildew and downy mildew (Parry, 1990). In the case of "compound interest" diseases the effect of inoculum level is transient because once the first infection is established, the pathogen produce more inoculum on the affected plants. This leads to the spread of the pathogen and development of the disease on other plants through out the crop leading to the severe epidemics provided there are favourable environmental conditions. Different relationships have been suggested between inoculum level and disease development. In the case of *Uromyces phaseoli* and *Phaseolus vulgaris* interaction, disease development was directly proportional to inoculum level with a constant lesion to spore ratio of 1:11 (Schein, 1964). In another study of the same interaction, Davison and Vaugham (1964) found an increase in disease as inoculum level increases up to certain stage until a maximum has been reached and then decrease. A similar trend was noticed for *Erysiphe graminis* on barley (Domsch, 1953). Since the different relationship has been suggested on the role of leaf age and inoculum level in the disease development this study aims to study their role in the broad bean rust disease.

MATERIALS AND METHODS

Experiment was carried out in the growth chamber of the biological sciences laboratory of the Wye College, UK in 1995. Fifteen treatment combinations were assessed for pustule production and spore production efficiency on potted broad bean plants in Complete Random Design (CRD) with four replication.

Preparation of plants

Plants for the experiment were grown in the College glass-house in "poly pots" of 10cm diameter. During the period of plant growth the mean, mean maximum and mean minimum temperature was 19.47, 29.1 and 14.1°C, respectively and an average day length of 16 hours. Pots were filled with the compost consisting four parts of Irish moss peat and one part of grit of 6mm or more diameter. The pH of the compost was adjusted to 6.5 by adding lime (CaO). Starter nutrient consisting Nitrogen (N), Phosphorus (P), Potassium (K), Mangnessium (Mg), Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn) and Molybdenum (Mo) was added to the compost to facilitate the plant growth. Pre-soaked seeds (2 to 5 seeds) were sown in each pot. Two plants were maintained in each pot after a week of germination. Plants were watered daily and a bio-control agent (*Amblyseius cucumeris*) was used to protect the plants from attack by thrips. Plants were kept in the glass house for about a month until they were ready for inoculation.

Preparation of inoculum

Uredospores of *Uromyces vicia-fabae* were collected from the Wye area and freeze dried at 3-5°C a month before the start of the project. Freeze dried uredospores were mixed with talc in the ratio of 1:50 and inoculate to 3 weeks old broad bean seedlings to produce fresh inoculum for this experiment. A regular supply of fresh inocula was made by inoculating new broad bean plants in succession at an interval of 15 days.

Inoculation

Five levels of inoculum; 1:50, 1:100, 1:500, 1:1000, 1:2000 were prepared by mixing fresh uredospore with acid washed talc (BDH) in w/w basis. All five inoculum were tested against three different aged leaves (young, middle-aged and old) of six week old plants having six fully expanded bifoliates and replicated four times. The lowest 2nd, 4th and 6th bifoliate were designated as old, middle-aged and young leaves respectively. A thoroughly mixed inoculum mixture was applied to the lower leaf surface of broad bean with the help of fine paint brush. Inoculated plants were lightly moisten by spraying with distilled water and immediately covered with a moistened polythene bag for next 24 hours to facilitate spore germination. Inoculated plant were then transferred to growth chamber by covering them with moistened plastic bags for first 24 hrs. Plant were kept in growth chamber kept at 20-22°C and illuminated for 16h/day at 12820 lx for sporulation for three weeks.

Data recording

Pustule production on each inoculated bifoliate was recorded from four, 1 cm^2 area marked at different positions on the upper surface of the leaf and total spore production by each bifoliate was weighed at an interval of 3 days from 8 days after inoculation (dai) to 20 dai. The relationship between pustule number and total spore production was studied using simple linear regression analysis.

RESULTS AND DISCUSSION

Effect of leaf age and inoculum level in pustule production

Differences were recorded in the number of pustule produced on different aged leaves. Young leaves produced the highest number of pustules followed by the middle-aged and old leaves irrespective of the inoculum levels (Fig. 1). This result suggests that contribution of individual leaf to the total pustule production varies according to its age. The highest pustule numbers were recorded in the leaves inoculated with the highest inoculum level (1:50 w/w) and a decreased pustule numbers were associated with decreasing inoculum level (Fig. 2).

The differences in the pustule number was not apparent at 8 dai however, the difference was pronounced in subsequent recordings where the standard inoculum level (1:50 w/w) yielded more pustules ($P < 0.05$) than other treatments. Other treatments were indifferent for this parameter. The interaction was noticed between leaf age and inoculum level for pustule numbers. Young leaves yielded significantly the highest posture number against standard inoculum (1:50) than other treatments (Fig. 3) and it was also the case with middle-aged leaves except at 8dai. In contrast the effect was minimal in the old leaves (Fig. 4).

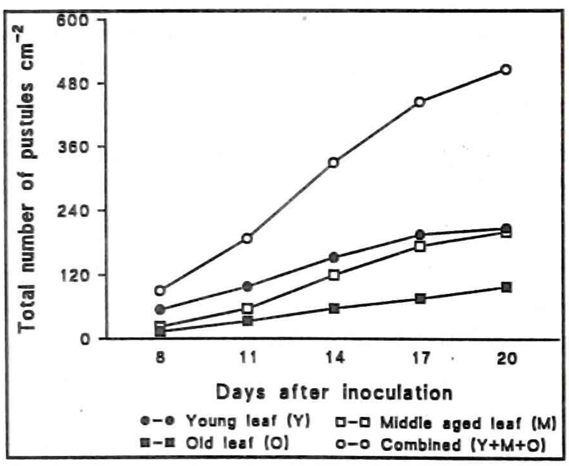

Figure 1: Leaf age vs pustule number over time

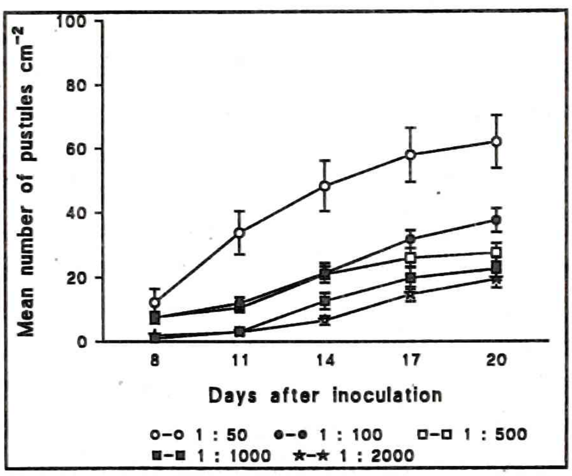

Figure 2: Inoculum level vs pustule number over time (bar indicate ± SEM)

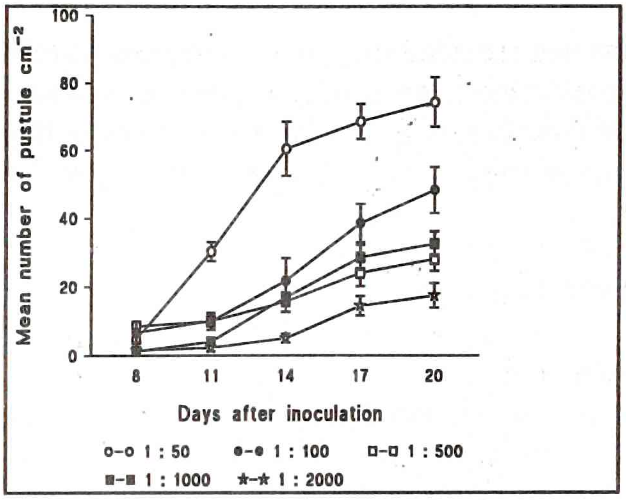

Figure 3: Inoculum level vs pustule number on young leaves

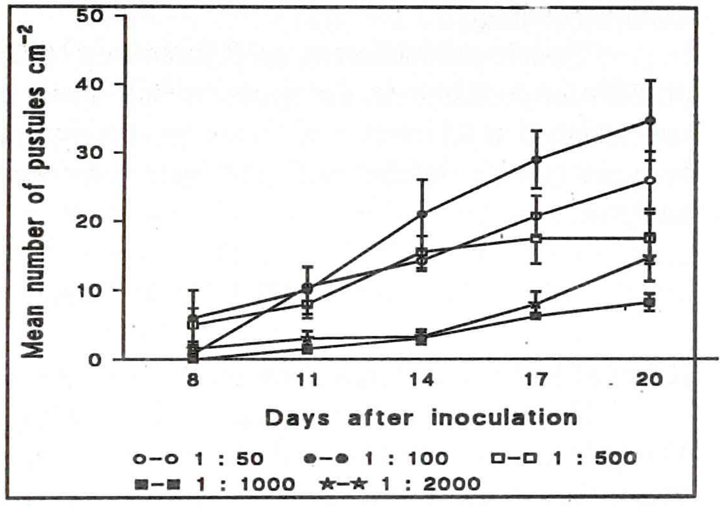

Figure 4: Inoculum level vs pustule number on old leaves

This study revealed an increased resistance of broad bean leaves to *U. viciae-fabae* with increasing leaf age. A similar effect of the leaf age was observed in susceptible coffee genotypes and *Hemileia vastatrix* where the resistance increased with leaf age. Pretorious *et al,* (1988) recorded an increased latent period as the adult plant resistant cultivar matured and little effect in the latent period in the susceptible cultivar in a wheat and yellow rust pathogen interaction. In the *Allium porrum - Puccinia allii* interaction, pustule density, pustule length and colony length decreased with increasing leaf age and there was a tendency for latent period to increase with increasing plant age (Jennings *et al.*, 1990). As found by Pretorious *et al,* (1988) in a susceptible *T. aestivum - P. recondita f.sp. tritici* interaction, very little effect was seen on latent period of *U. viciae-fabae* in different aged leaves however a distinct variation was recorded in the pustule density as found by Jennings *et al.,* (1990) for *A. porrum -P. allii* interaction.

The difference in the pustule production between different aged leaves in broad bean may be due to the differential germination rate of the uredospores on different aged leaves. The uredospore germination of *P. striiformis* on adult *T. aestivum* leaves with durable resistance showed a lower germination rate on these than on younger leaves (Rossel, 1976). Bock (1962) and Nutman and Roberts (1963) reported that *H. vastatrix* uredospore germination is greater on young than on intermediate and old coffee leaves. In some plant-pathogen interactions Eskes and Toma Braghini (1982) found the highest uredospore density in the youngest leaves which declined rapidly with increased leaf age when inoculated with same inoculum. The presence of toxic material produced by the resident micro flora or mycoparasites on the leaf surface of older leaves might prevent the germination of uredospores. Martin *et al.* (1986) have shown the role of non pathogenic fungi induced host metabolites with antifungal activity and reduced germination of *H. vastatrix* uredospores. Such metabolites were more abundant on the older leaves than on younger ones. Increased frequencies of the attachment of the spores , a high percentage germination, more penetration coupled with rapid colonisation of intercellular space in the young leaves could have produced more pustule than on middle-aged and old leaves. Another possible reason for the reduced pustule production on old aged leaves may be the poor nutritional relationship between pathogen and physiologically less active, senescing old leaves.

Effect of leaf age and inoculum level on spore production efficiency

The amount of spore production was the highest on young leaves and was followed by middle aged and old leaves (Fig 5). The standard inoculum level produced the highest amount of spores than other inoculum levels on young and middle-aged leaves. Spore production per pustule was the highest at the beginning of sporulation i.e. 8dai irrespective of leaf age and inoculum level and decreased gradually as the number of pustules increased over time.

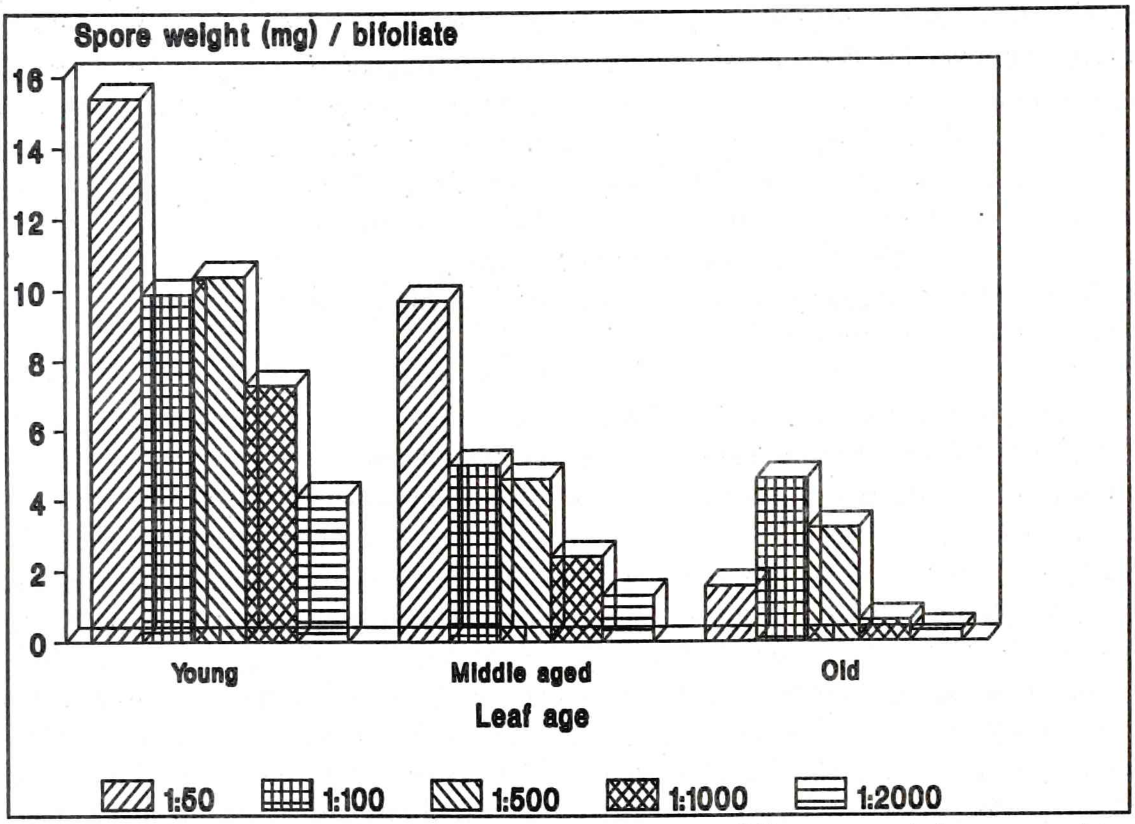

Figure 5: Spore production during 11-14 days at different aged leaves against inoculum levels

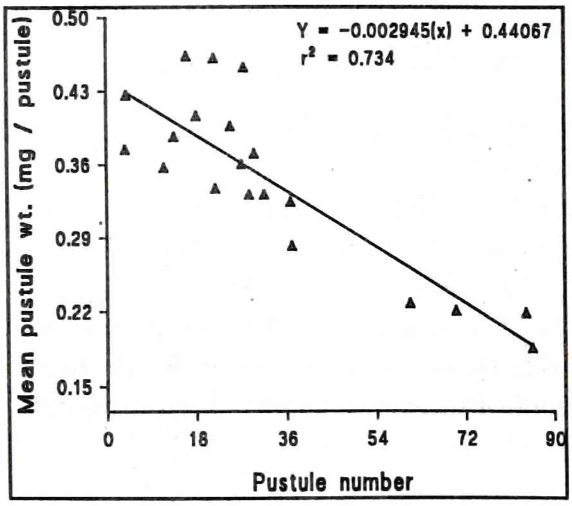

Figure 6: Pustule number and spore weight on young leaves

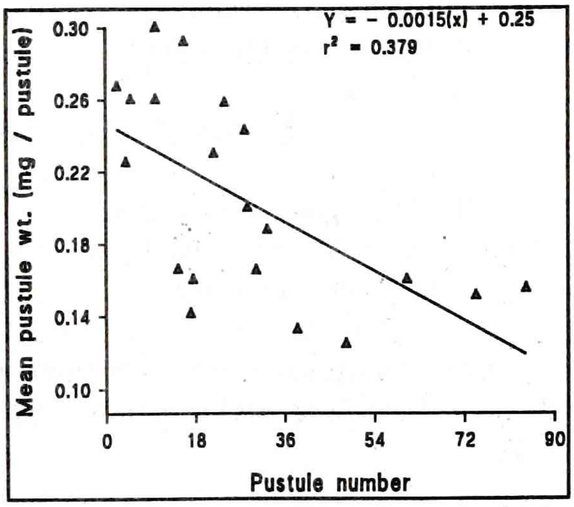

Figure 7: Pustule number vs spore weight on middle aged leaves

Regression analysis of the data suggested an inverse relationship between pustule number and spore production efficiency (spore weight/pustule) for young and middle-aged leaves (Figs. 6 and 7).

Correlation coefficient (r^2) for the young and middle-aged leaves was 0.734 and 0.379 respectively. Regression for both aged leaves was significant ($P<0.001$). There was no clear relationship between pustule number and spore weight per pustule in old-aged leaves.

An increased pustule numbers was associated with increased level of inoculum in this study. However there was no difference in time for appearance of the pustule and sporulation between leaves inoculated with different inoculum levels. Production of fewer, isolated pustules on the leaves inoculated with low inoculum level suggest some degree of restriction in intercellular growth of the fungus. Such restriction might be due to activated plant response which might be effective only against low inoculum level since a susceptible plant does not mean absolutely susceptible. Leaf area has also a grater role in the sporulation time, which is mostly followed by complete colonisation of tissues. Synchronisation in the time required to colonise available tissues in leaves inoculated with low and high inoculum level might be the reason for uniformity in sporulation time.

Availability of nutrients play an important role in the growth, development and reproduction of plant pathogenic fungi. Sporulation of plant parasitic fungi frequently accompanies a decrease in available nutrients; however the reduction in the nutrient may not be direct stimulus for formation of reproductive structure (Moore-Landecker, 1972). In this interaction between broad bean and its rust pathogen, leaves inoculated with high inoculum level resulted more numbers of smaller sized pustules than those inoculated with low inoculum levels. Such consequence might be due to many intercellular hyphae competing for limited nutrients available in leaves inoculated with high inoculum level. In contrast, fewer intercellular hyphae to share nutrients available in leaves inoculated with low inoculum level made them access to more nutrients for producing big pustules with more sporulation. Madelin (1956) found the size and weight of the basidiocarp produced by *Coprinus lagopus,* proportional to the quantity of the media available. When cultured in the small amount of media, it produced smaller sized basidiocarp with less basidia. If there is sufficient nutrients available, the space availability on the leaves might have significant role in spore size. Crowed infection hyphae could not form big pustule because they are hemmed by their neighbours for the leaf area. No relationship between pustule number and sporulation efficiency in old-aged leaves suggests more influence of the leaf physiology (senescence) rather than factors studied.

CONCLUSION AND RECOMMENDATION

Both leaf age and inoculum level were important factor in broad bean - broad bean rust pathogen interaction and subsequent disease development. This study has shown that the younger leaves are more prone to the disease than the older ones. Similarly the highest inoculum level (1:50 w/w) was associated with high level of disease. An obvious interaction was noted between leaf age and inoculum level for the disease development. These results have important significance on the strategic application of control measures against broad bean rust disease.

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REFERENCES CITED

Bock, R.K. 1962. Dispersal of uredospores of *Helmileia vastatrix* under field conditions. Transactions of the British Mycological Society 45: 63-74.

Coutinho, T.A., F.J.H. Rijkenberg, and M.A.J. Asch van. 1994. The effect of leaf age on infection of Coffea genotypes by Helmileia vastatrix. Plant pathology 43: 97-103.

Davison, A.D. and E.K. Vaughan. 1964. Effect of uredospore concentration on determination of race of Uromyces phaseoli var. phaseoli. Phytopathology 54: 336-338.

Domsch, K.H. 1953. Uber den Einflusz photoperiodischer Behandlung auf die Befallsintensitat beim Gerstenmehltau. Arch. Mikrobiol. 19: 287-318.

Eskes, A.B. and M. Toma-Braghini. 1982. The effect of leaf age on incomplete resistance to Helmileia vastatrix. Netherlands journal of plant pathology 88: 215-126.

Guyot, A.L. 1957. Les Uredinees (ou ruuilles des vegetaus) III. *Uromyces*. Encyl. Mycol. 29: 505-554.

Hyde, P.M. 1977. The effect of leaf age on infection of wheat seedlings by *Erysiphe graminis* and on subsequent colony development. Phytopathologische Zeitschrift, 88: 299-305.

Ibrahim, A.A., A.M. Nassib and M. El-sherbeeny. 1978. Production and Improvement of grain legume in Egypt. In G. C. Hawtin and G. J. chancellor. eds. Food legume improvement and development. ICARDA and IDRC, Aceppo, Syria. pp 39-46.

Jennings, D.M., B.V. Fold-Lloyd and G.M. Bulter. 1990. Effect of plant age, leaf position and leaf segment on infection of leek by leek rust, *Puccinia allii*, Plant Pathology 39: 591-597.

Launden, G.F. and J.M. Waterston. 1965. *Uromyces appendiculatus* CMI Descriptions of pathogenic Fungi and Bacteria, No. 57. CMI, UK.

Madelin, M.F. 1956. Studies on the nutrition of *Coprinus lagopus* fr, especially as affecting fruiting, Ann. Bot. Lond. II, 20: 307-330.

Martins, E.F.M., D.S. Roverattii and W.B.C. Moraes. 1986. Elicitation of stress metabolites in coffee leaves by a non pathogen. Fitopatologie Brasileira, 11: 683-695.

Moore-Landercker, E. 1972. Fundamentals of the fungus. Printice Hall International, London, pp 234.

Nutman, F.J. and F.M. Roberts. 1963. Studies on the biology of *Helmileia vastatrix* Berk. and Br. Transactions of the British Mycological Society, 46: 47-48.

Parlevliet, J.E. 1975. Partial resistance of barley to leaf rust, 1. Effect of cultivar and development stage on latent period. Euphytica 24: 21-27.

Parry, D. 1990. Plant pathology in agriculture. Cambridge University Press, Cambridge, pp 63-68.

Pretorius, Z.A., F.H.J. Rijkenberg and R.D. Wilcoxson. 1988. Effect of growth stage, leaf position and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. Plant pathology 37: 36-44.

Russel, G.E. 1976. Germination of Puccinia striiformis *uredospore* on leaves of adult winter wheat plants. Annals of applied biology, 82: 71-78.

Schien, R.D.1964. Design, performance and use of a quantitative inoculator. Phytopathology, 54: 509-513.

Williams, P.F. 1978. Growth of broad beans infected with Uromyces viciae-fabae. Ann. Appl. Biol., 90:329-334.