EPIDEMIOLOGY OF LENTIL RUST IN ETHIOPIA WITH SPECIAL REFERENCE TO DISEASE PROGRESS AND YIELD LOSS ASSESSMENT

By

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This work is dedicated to my mother, Yeshi G/Mariam, my wife Azeb and our daughters

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"Commit to the LORD whatever you do, and your plans will succeed."

Proverbs 16:3

"I will praise you, O LORD, with all my heart; I will tell of all your wonders."

Psalm 9:1

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PREFACE

"Epidemiology is the science of disease in populations."

J.E. Van der Plank

"The preface is that part of the book which is placed first, written last and read least."

Alfred Lokta

This thesis is comprised of several investigations compiled into six chapters each of which is prepared in article format with the view of publishing it in a scientific journal. Chapter 1 deals with an overview of lentil rust symptoms, economic importance, taxonomy, geographic distribution of the disease, host range, physiologic races, mechanism of attack, epidemiology, disease management and a conclusion with suggestions for future research in Ethiopia and priority areas that may help bridge the lentil rust research gap in a broader context.

Chapter 2 is about sequential analyses of lentil rust epidemics by means of cross-sectional and longitudinal studies whereby the different disease and crop responses to levels of stimulus were evaluated in a cropping season. The dynamics of lentil rust epidemics in relation to crop growth is one of the subjects of this chapter. This chapter also reports on the results of yield loss assessments and yield loss prediction models.

Chapter 3 deals with the determination of nitrogen, phosphorus and crude protein concentrations of seeds and straws harvested from lentil plants with varying levels of rust infection. Livestock production is an integral part of farming systems in Ethiopia, therefore results of dry matter degradability of lentil straws have been included and their implications have been discussed.

Chapter 4 touches upon an inoculation technique that can be used for quantitative lentil rust experiments under glasshouse or controlled environmental conditions. Although not in line with technique development, Chapter 5 contracts with the environmental conditions affecting urediniospore germination and rust development on lentil. This chapter reports on the optima for accurate resistance phenotyping.

Chapter 6 is a dossier of a resistance study. Five lentil genotypes were used for this study, each with a different level of resistance. The chapter reports on components of rust-resistance that were used to compare the test genotypes. In addition, selection criteria for use in resistance breeding have been recommended.

Chapter 7 describes the response of a resistant and susceptible lentil cultivar, at histological level, to rust infection.

CHAPTER 1

A RETROSPECTIVE UNDERSTANDING OF LENTIL RUST

"Chemical industry and plant breeders forge fine technical weapons; but only epidemiology sets the strategy… against plant disease."

J. E. Van der Plank

INTRODUCTION

Lentil (*Lens culinaris* Medikus) is one of the major cool-season food legumes grown in many parts of the world (Cubero, 1981; FAO, 2004). The highest producing (top 12) countries in 2003 were, in order of production India, Turkey, Canada, Australia, Syria, Nepal, China, Bangladesh, USA, Iran, Ethiopia and Morocco. Major lentil producing countries are listed in Table 1.1. Ethiopia is the single largest producer of lentil in Africa accounting for ca. 47% of the total production on the continent (FAO, 2004).

Lentil is an important component of farming systems in many countries worldwide (Westphal, 1974; Erskine and Ashkar, 1993). Lentil enrichs soil fertility through N_2 fixation and green manuring (Saxena, 1981). It serves as a source of dietary protein and other essential micronutrients in human nutrition in many developing countries. The seed (split and raw) have the following approximate composition: moisture 14.2%, protein 26.4%, fat 0.8%, and ash 2.6% (Abu-Shakra and Tannous, 1981). The lysine content, an amino acid essential for the human body, of raw dry lentils is 600 mg/100 g N. This is the highest when compared with other food legumes, and cereals, the latter which are staple foods in many of the poorer lentil producing countries, are deficient in lysine. As opposed to other food legumes, few anti-nutritional factors are reported (Nygaard and Hawtin, 1981). Moreover, lentil straw and residues from threshing are excellent livestock feed.

Diseases are known to affect growth and yield of lentils. Of these, rust caused by the fungus *Uromyces viciae-fabae* (Pers.) Schroet. is potentially damaging to lentil crops, has a wide distribution (Laundon and Waterston, 1965), and limits production of the crop in many countries (Khare, 1981; Beniwal *et al.*, 1993). Previously, there has been much

interest in biological and pathological aspects of the fungus, and management of the disease, particularly through host plant resistance. A general review of lentil rust was published by Bayaa and Erskine (1998).

From the literature, it is apparent that considerable information on the disease and the pathogen exists. Quite recently, environmental effects on *U. viciae-fabae* development, the mechanism by which the pathogen attacks its hosts, effects of the pathogen on its host's physiological functions and some aspects of infection processes and crop loss models have been studied and reported. However, many questions relating to lentil rust epidemiology exist. Controversy also exists with respect to host specialization. In this chapter, a detailed account of the current state of research on lentil rust and its incitant, *U. viciae-fabae,* is provided. I also assess research findings thus far with the aim of making suggestions about future research that may contribute to improved lentil rust management.

RUST SYMPTOMS

The rust pathogen attacks all aerial plant parts. Yellowish-white spermagonia and aecial cups develop on the abaxial surface of leaflets and pods. The aecia are borne singly or arranged in a circular manner as small groups on leaflets (Fig. 1.1). They eventually turn light brown before brown uredinia, circular to oval in shape (diam.~ 1 mm), develop on both surfaces of the leaves, stems and pods (Khare, 1981). Pustules are powdery and may coalesce with each other (Fig. 1.1). Later in the season telia are formed from the same mycelium mainly on stems and branches of the plant (Agrawal and Prasad, 1997). Telia are firm in texture, raised and black in colour.

ECONOMIC IMPORTANCE

Lentil rust is economically important in many regions of the world, namely Africa, Asia, and Latin America. The disease is particularly important in sub-Saharan Africa (Bejiga, Yohannes and Knight, 2000), being the major biotic constraint to production in Ethiopia and Morocco (Johansen *et al.*, 1994).

Seed yield loss in lentils attributable to rust has been estimated at 25% in Ethiopia (DZARC, 1993). In 1997, however, a lentil rust outbreak throughout Ethiopia caused yield losses of up to 100%. Evidence in this regard was provided by nearly 2500 ha of lentil being completely wiped out by rust in the Gimbichu district of Ethiopia. This resulted in a financial loss of one million U.S. dollars (Negussie, Bejiga and Million, 1998). Figure 1.2 shows a severe rust outbreak on a farmer's lentil crop in central Ethiopia.

The disease is a constraint to lentil production in India, Pakistan and Bangladesh (Agrawal, Singh and Lal, 1993; Ilyas, 1993; Bakr, 1993). In India, for instance, a crop loss of 100% has been reported (Khare, 1981). Lentil rust is also important in Latin American countries such as in Chile (Bascur and Sepulveda, 1989).

There is little evidence of any national or regional crop loss assessment programmes for lentil rust. Occasionally, intellectual guesses have been made whenever a rust outbreak occurs. Singh and Jhooty (1986) attempted to develop a damage function, specifying an 11.5-kg lentil seed yield reduction per ha with a 1% increase in rust severity. The function reported by Singh and Jhooty (1986) emphasizes the importance of the disease, but their experimental results have several shortcomings in estimating yield losses even within the geographic area where the loss assessment experiment was

conducted. In order to develop a reliable method for translating rust measurements into seed yield loss in lentil, several experiments need to be conducted in the geographic area of interest for at least three years, under the range of conditions found under normal farming practices (James, 1974).

Singh and Jhooty (1986) have developed a critical (single) point yield loss model with a high coefficient of determination ($R^2 = 0.96$). This indicates that lentil yield loss (damage) is primarily a function of rust severity at a particular growth stage. However, no mention was made of the development stage of the crop or the critical time during the crop's life cycle at which the function can provide an estimate of yield loss for a given rust severity. For example, Sache and Zadoks (1995a) suggested the possibility of predicting the effect of rust (*U. viciae-fabae*) on yield components of faba bean by a critical point model using disease severity assessed on the middle or bottom canopy layer in the mid-flowering stage of the crop. Moreover, it is not clear whether or not the model of Singh and Jhooty (1986) can be used to estimate the loss for the whole epidemic and can give the same estimates of loss at different growth stages. For instance, Brooks (1972) showed the impossibility of correlating loss in yield of mildew-infected spring barley early in the season with severity of mildew at a later growth stage.

BIOLOGY OF THE LENTIL RUST FUNGUS

Cummins and Hiratsuka (1983) recognized five basic life cycle variations of the rust fungi, and according to them *U. viciae-fabae* may be categorized under the one exhibiting an automacrocyclic life cycle. That means, it has: (1) all the five spore states (spermagonial, aecial, uredinial, telial and basidial), and (2) no alternate hosts or it is a

non-host alternating type. Unlike *Puccinia graminis* (stem rust of cereals), the aecial and telial states of *U. viciae-fabae* occur on the same host plant. Although *U. viciae-fabae* is autoecious, it has collateral (alternative) hosts (Table 1.2).

In general, *U.viciae-fabae* goes through the different stages of development that commonly occur in the sub-division basidiomycotina (basidiomycetes). The sexual spores [monokaryotic spores (basidiospores and spermatia)] give rise to dikaryotic (N + N or containing two sexually compatible nuclei) and non-repeating (asexual) spores called aeciospores. The latter, upon germination produce dikaryotic mycelia, which in turn produce urediniospores (repeating vegetative spores). Urediniospores ultimately give rise to telia and teliospores (basidia-producing spores) spores (Cummins and Hiratsuka,1983).

The sexual cycle usually occurs only once in a single crop-growing season, whereas, the asexual cycle takes place many times during a growing season. The former is known as the annual sexual cycle and the latter as a repeating asexual cycle (Zadoks and Schein, 1979). Therefore, the sexual cycle alternates with a number of asexual cycles.

Commonly, the telial state has a survival value. For example, teliospores produced by a telium permit *U. viciae-fabae* to survive unfavourable periods; moreover, these teliospores can germinate as soon as they are fully formed without going into dormancy (Prasada and Verma, 1948). Upon germination, teliospores produce basidia and basidiospores capable of infecting lentils and starting the annual life cycle (infection cycle). This unique feature of the teliospores plays an important role in the epidemiology of lentil rust.

TAXONOMY

Uromyces viciae-fabae (Pers.) J. Schroet. has the following synonyms: (1) *Uredo viciae-fabae* Pers., (2) *Uromyces fabae* (Pers.) De Bary, (3) *Uromyces orbi* (Pers.) Fuckel., (4) *Uromyces viciae* Fuckel., (5) *Uromyces polymorphus* Peck. and Clint., and (6) *Uromyces yoshinagai* P. Henn. (Laundon and Waterston, 1965).

As mentioned, five types of spore bearing structures, namely the spermagonium (plural spermagonia), aecium (plural aecia), uredinium (plural uredinia), telium (plural telia) and basidium (plural basidia), are recognized and respectively denoted by the Roman numerals O, I, II, III, IV, and V (Cummins and Hiratsuka, 1983; Preece and Hick, 1990; Agrios, 1997). Characteristic features such as morphology, colour and size of these spore-bearing structures (states) and their respective spores are important taxonomic features and often used to differentiate rust fungi.

According to Arthur (1962), Laundon and Waterston (1965), and Cummins (1978), the following features distinguish *U. viciae-fabae*:

1. Spermagonia mostly on abaxial leaf surface, amphigenous in small groups associated with aecia.
2. Aecia (Fig. 1.3A) mostly on abaxial leaf surface in small groups, predominantly along veins surrounding the spermagonia or sometimes scattered, peridium cupulate, whitish, 0.3-0.4 mm diam.; aeciospores 18-26 x 15-21 μm, broadly ellipsoid, wall hyaline (colourless), finely verrucose and 1-1.5 μm thick (Fig. 1.3B).
3. Uredinia (Fig. 1.3C) amphigenous, yellowish brown (cinnamon), 0.5 mm diam; Urediniospores 22-32 x 17-25 μm, broadly ellipsoid, wall light golden brown, 1-2.5

μm thick, uniformly echinulate (Fig. 1.3D), pores three to five, equatorial or occasionally scattered.

4. Telia (Fig. 1.3E) sometimes on adaxial surface or sometimes amphigenous and on stems, exposed, blackish brown, compact and 1-2 mm diam; Teliospores ellipsoidal, obvoidal or cylinderical, rounder or subacute above, 24-40 x 17-25 μm; wall chestnut-brown, smooth (Fig. 1.3F), 1-3 μm thick at the sides, 5-12 μm thick at the apex, pedicels brownish at least apically, up to 100 μm long.

GEOGRAPHICAL DISTRIBUTION

Uromyces viciae-fabae is a cosmopolitan fungal species due to its worldwide distribution (Arthur 1962; IMI, Distribution Map of Plant Diseases Map No. 200, 1990). The fungus is reported from India (Prasada and Verma, 1948), Australia, Mexico, New Zealand (Arthur, 1962), Ethiopia (Stewart and Dagnachew, 1967), Canada (McKenzie and Morall, 1975), Algeria, Argentina, Bulgaria, Chile, Cyprus, Egypt Iran, Italy, Morocco, Pakistan, Portugal, Syria and Turkey (Beniwal *et al.*, 1993), and Nepal (Karki, 1993). For distribution regions, see Fig. 1.4.

HOST RANGE AND SPECIFICITY

U. viciae-fabae has a wide host range (Arthur, 1962). It attacks several genera belonging to the Leguminosae (Fabaceae). These include *Lens*, *Lathyrus*, *Pisum* and *Vicia* (Laundon and Waterston, 1965). For more information, see Table 1.2.

The fungus is not species-specific (host-specific). Conner and Bernier (1982a), studying isolates of the pathogen collected from *Pisum sativum* L., nine species of *Vicia*

and seven species of *Lathyrus* at numerous locations in Canada, found no marked difference in host specificity. There is however a difference among isolates in their ability to infect a given species. For example, a Manitoban isolate from *P. sativum* infects lentil, whereas the Quebec isolate does not (Conner and Bernier, 1982a). These findings suggest that the concept of host specificity or *formae speciales* (Gaumann, 1934) may be less important now than previously believed. Moreover, Conner and Bernier (1982a) expressed concern that studies used for *formae speciales* identification were not systematic and complete enough.

In their host range studies, Kapooria and Sinha (1966) reported that *Lathyrus aphaca* was resistant to the pathogen, but later the same host species (*L. apaca*) was characterised as susceptible (Kapooria and Sinha, 1971). This implies that the prevailing environment influences the number of host species attacked by *U. viciae-fabae* at any one time. Nevertheless, several possibilities might be ascribed to the different results obtained by these workers. Poor infection techniques could be one possible factor responsible for the inconsistent reaction of the host to the pathogen.

In addition to the above-mentioned possibility, spatial variation in the determination of host spectrum of the pathogen population cannot be ruled out. This was evidenced by the differences observed between Canadian and European isolates of the fungus (Conner and Bernier, 1982a).

PHYSIOLOGIC RACE IDENTIFICATION

Physiologic races (race) as a taxon of *U. viciae-fabae* are characterised by specialization to different cultivars of one host species (FBPP, 1973). The history of race identification

in *U. viciae-fabae* started as far back as the 1930s. Hiratsuka (1933) and Kispatic (1950) reported the early variability studies on *Uromyces viciae-fabae*. Later, several reports were published on race identification or variation in pathogenicity within *U. viciae-fabae* (Singh and Sokhi, 1980; Conner and Bernier, 1982a; Singh *et al.*, 1995). Although our present knowledge about physiologic races of *U. viciae-fabae* is incomplete, the number of races reported so far ranges from five to 11 (Table 1.3).

Race identification was based on the use of a given set of differentials. Singh and Sokhi (1980) found that some lentil lines are suitable differentials for race identification, but there is no further evidence supporting this finding. Mostly, the set of differentials used for classifying the races reported to date, varied in different studies. This indicates that the system of race identification in *U. viciae-fabae* has not been standardized. In conclusion, current evidence shows that a standard set of differentials and an across-the-board race designation system for *U. viciae-fabae* are lacking. Future endeavours on developing a working system of race analysis by all concerned will help to bridge this gap.

DISEASE DEVELOPMENT AND EPIDEMIOLOGY

Disease development

Contact between the cell surfaces of a pathogen and its host is a pre-requisite for successful establishment of a pathogenic relationship. Adhesion of the two surfaces, a pre-penetration phenomenon, is of basic importance to the subsequent sequence of events in disease development (Beckett, Tatnell and Tylor, 1990). Substances present in the extracellular matrix produced by many pathogenic fungi (Hamer *et al.*, 1988) facilitate

adhesion or attachment of germlings and ungerminating spores. Such a matrix has been reported for *U. viciae-fabae* (Woods and Beckett, 1987; Beckett and Porter, 1988). The presence of mucopolysaccharides in the urediniospore wall matrix of *U. viciae-fabae* has been detected and these are thought to contribute towards the attachment of the spore to a host surface (Woods and Beckett, 1987). More recently the exudation of extracellular matrix materials in association with ungerminating and germinating urediniospores of *U. viciae-fabae* on host and synthetic surfaces was confirmed (Beckett *et al.*, 1990). Moreover, Deising and Mendgen (1992) have presented evidence that urediniospores of *U. viciae-fabae* produce serine-esterases, which possibly assist in adhesion of spores to the host surface.

In addition to extracellular matrix substances, morphological features of germinating urediniospores such as adhesion pads are known to assist attachment of the spores to the host surface by increasing the area of contact with the substratum (Clement *et al.*, 1997). For example, urediniospores of *U. viciae-fabae* form this adhesion pad on their host surface when they are fully imbibed and germinating (Clement *et al.*, 1997).

Disease is the result of an interaction among factors of a pathogen (source and amount of inoculum, virulence, etc.), host (susceptible cultivar, right stage of growth), environment (favourable conditions for disease development), time (frequency and duration of favourable events) and human activities (farming practices). The perfect synchronization of all these components results in the appearance of a certain disease. For example, infected plant debris mixed with lentil seeds or residues in crop fields are believed to act as the primary source of inoculum (Khare, 1981). However, collateral hosts of *U. viciae-fabae* may also serve as sources of inocula. According to Kramm and

Tay (1984), the amount or concentration of inoculum that is best to produce infection at the seedling stage is 3 x 10^4 urediniospores ml^{-1} provided that the inoculated plants are exposed to humid conditions (ca. 100%) for one to four days.

In addition to the above, susceptible lentil cultivars are more vulnerable to rust attack at the flowering stage than at vegetative stages (Khare, 1981). As opposed to this, Kramm and Tay (1984) reported that plants at the seedling stage are also highly susceptible. The influence of the crop's age on disease development therefore still requires investigation. Finally, high relative humidity, cloudy and drizzling weather with a temperature of 20 to 22°C favour rust development (Khare, Bayaa and Beniwal, 1993).

Epidemiology

Lentil rust epidemics generally occur annually in some lentil growing countries. Such localities are referred to as hotspots, and these include Akaki in Ethiopia, Ishurdi in Bangladesh and Pantnagar in India (Erskine *et al.*, 1994a; 1994b). Meteorological variations, management practices, combined with cultivar resistance and a changing pathogen population, result in significant variation in severity of yearly rust epidemics (Eversmeyer and Kramer, 2000). For example, a countrywide and severe epidemic in Ethiopia occurred in 1997 (Negussie *et al.*, 1998). This epidemic occurred as a consequence of Elniño which created weather conditions wetter than the normal growing season (NOAA, 2002). Susceptible local landraces (farmer's varieties) grown on large areas throughout the country also contributed to the epidemic. These landraces were destroyed in most parts of the country and yield reductions of up to 100 % were measured (Fig 1.2).

Epidemiological processes

Sache and Zadoks (1995b) studied the epidemiological aspects of rust caused by *U. viciae-fabae* in a controlled environment (18°C). They used the highly susceptible faba bean cv. Alfred. According to them, the latent period is ca. eight to 10 days, an infection efficiency of 0.11 lesions per inoculated spore occurred, and a spore production capacity of 4.3×10^4 (for the first leaf) and 9.3×10^4 (for the second leaf) spores per lesion, were noted. In the lentil-rust pathosystem, however, no attempt has been made so far to quantify these processes. Undoubtedly, the availability of information on latent period, infection efficiency and spore production capacity in lentils will be helpful to breeding programmes for rust resistance.

***Environmental effects on lentil rust caused by* Uromyces viciae-fabae**

Abiotic environment

Effects of abiotic environmental components such as water, temperature and radiation on plant disease development are well documented. Germination of urediniospores of rust fungi is affected by moisture duration, temperature and quality of light (daylight and artificial) (Calpouzos and Chang, 1971; Chang, Calpouzos and Wilcoxson, 1973; Kochman and Brown, 1976; Knights and Lucas, 1981; Subrahmanyam, Reddy and McDonald, 1988; Joseph and Hering, 1997).

Joseph and Hering (1997) reported that urediniospores of *U. viciae-fabae* germinate well in the temperature range from 5 to 26°C, with optimum germination at 20°C. Infection of leaves by urediniospores of *U. viciae-fabae* progressively increases as

leaf wetness period increases, and the lower the temperature, the slower the infection process (Joseph and Hering, 1997).

According to Prasada and Verma (1948), aeciospores of *U. viciae-fabae* germinate at 17-22°C. After infection, secondary aecia are formed if the prevailing temperature is similar to the above or uredinia are produced at 25°C at a later crop stage. Aeciospores thus play a major role in the dissemination of lentil rust. In the highlands of Ethiopia, the temperature rises only at the end of October by which time urediniospores are formed. Though the urediniospores are of short duration in comparison with aeciospores, they will repeat themselves resulting in several infection cycles before the cropping season is over.

According to Prasada and Verma (1948), urediniospores germinate best (70-80% germination) at 17-18°C. Towards the end of the season, at a later stage of disease development, dark brown to black and elongated telia are formed mainly on stems and branches. Telia will form teliospores which can readily germinate, if conditions are favourable, to form basidia and basidiospores capable of initiating a new epidemic. Therefore, teliospores associated with host plant debris can provide a means for infection of newly sown lentil plants the following season which allows lentil rust to begin its cyclic development. Although temperatures ranging from 12°C to 22°C favour the germination of teliospores, the optimum temperature is 17-18°C.

The infection by rust is also dependent on light as different species of rust fungi vary in their responses to light. In *U. viciae-fabae*, light inhibits urediniospore germination. Light also inhibits the germination of urediniospores of such rust species as *Puccinia graminis* f. sp. *avenae* and *P. coronata* f. sp. *avenae*, but the inhibition of spore

germination caused by light is irreversible (Kochman and Brown, 1976). Contrary to this, in species such as *P. arachidis* (Subrahmanyam *et al.*, 1988) and *U. viciae-fabae* (Joseph and Hering, 1997) the inhibition phenomenon is reversible. *U. viciae-fabae* urediniospores in which germination has already been inhibited by light can resume germination immediately after being placed in the dark for 40 min at 20 °C (Joseph and Hering, 1997).

Biotic environment

Several bacteria and yeast species have been found in association with various fungal pathogens including *U. viciae-fabae* (Fokkema and Van Der Meulen, 1976; Doherty and Preece, 1978). Parker and Blakeman (1984b) reported several species of microflora that are associated with *U. viciae-fabae* on infected leaves. These include bacteria (*Pseudomonas* spp.), yeasts (*Sporobolomyces* spp. and *Cryptococcus* spp.), *Penicillium* spp. and *Trichoderma viride*.

The effects of microorganisms as mentioned above in the disease environment may be positive, negative or neutral (Zadoks and Schein, 1979). Growth of germ tubes of *U. viciae-fabae* urediniospores is greatly stimulated in the presence of *Cryptococcus* and *Sporobolomyces* yeasts (Parker and Blakeman, 1984b). The former yeast also increases urediniospore germination and infection (Parker and Blakeman, 1984c). Inoculation of leaves with *Cryptococcus* cells 24 h before the addition of *U. viciae-fabae* urediniospores enhances infection (Parker and Blakeman, 1984c).

Trichoderma viride and *Penicillium* sp., although to a lesser extent than the yeasts, also stimulate growth of urediniospore germ tubes (Parker and Blakeman, 1984b).

Bacterial species on the other hand either reduce growth of germ tubes or prevent germination of urediniospores (Parker and Blackeman, 1984b). Clearly, the effects of these phylloshpere microorganisms may have implications on epidemiological processes, outbreaks and biological control of rust caused by *U. viciae-fabae*.

Plant pathogenic fungi are known to live not only in association with non-pathogenic microorganisms, but also with other disease causing agents. In line with this, different pathogens often occur together in the same crop and may infect the same plant. Lentil, for instance, is infected by a number of pathogens including viruses (Bayaa and Erskine, 1998). Interactions between viruses and rusts may increase host susceptibility (Beniwal and Gadauskas, 1974), or decrease host susceptibility to rust infection (Potter, 1982). Some virus infections (bean yellow mosaic virus and bean leaf roll virus) have been reported to decrease susceptibility to infection by *U. viciae-fabae* as evidenced by decreased pustule density (Omar *et al.*, 1986).

MECHANISM OF ATTACK BY *UROMYCES VICIAE-FABAE*

Except for the urediniospores of the soybean rust fungus *Phakopsora pachyrhizi*, and the tree rusts *Puccinia psidii* and *Ravenelia humphreyana*, which infect their hosts through the cuticle (Hunt, 1968; Bonde, Melching and Bromfield, 1976), rust fungi usually enter their host through natural openings such as stomata (Deising and Mendgen, 1992). Urediniospores give rise to a series of infection structures such as germ tubes, appressoria, substomatal vesicles, infection hyphae and haustorial mother cells, which in turn form haustoria (Mendgen *et al.*, 1988). The latter are formed within the host cell

after entering the cell without causing lethal injury (FBPP, 1973; Deising and Mendgen, 1992).

It is generally assumed that the haustorium is an organ by which a fungus absorbs nutrients from the host cells (FBPP, 1973). However, the haustorium of *U. viciae-fabae* acts as an essential structure for the biosynthesis of metabolites such as thiamine in addition to nutrient uptake (Sohn *et al.*, 2000).

U. viciae-fabae enters its host through stomata. Beckett *et al.* (1990) demonstrated the formation of an appressorium of *U. viciae-fabae* over a stoma. Pre-penetration behaviour of stomate-entering rust fungi is characterized by responses to chemical stimuli (chemotropisms) and/or surface stimuli (thigmotropisms or contact tropisms) such as ridges around stomata (morphological features of the stomatal guard cells) (Wynn, 1981; Wynn and Staples, 1981; Deising and Mendgen, 1992). Moreover, host recognition by rust fungi is also assisted by tropisms (Wynn, 1981). Deising and Mendgen (1992) studied the pre-penetration behaviour of *U. viciae-fabae* on an artificial membrane that mimics thigmotropic signal of the stomatal pore, and the fungus responded by forming infection structures.

Directional growth is one of the five specific tropisms on which urediniospore germ tubes of stomate-entering fungi depend to get them to the point where they can initiate infection (Wynn, 1981). For example, gradients in pH at the leaf surface influence the direction of germ tube growth of *U. viciae-fabae* (Edwards and Bowling, 1986).

After rust fungi, including *U. viciae-fabae*, have entered their hosts, they still have to overcome the cell wall that functions as a physical barrier and establish biotrophy. Plant cell walls can act as physical defense barriers to pathogen attack (Frittrang, Deising

and Mendgen, 1992). These walls consist of pectic substances and cellulose. The former constitute a large proportion of the primary cell wall and the latter is the main component of the secondary cell wall (Agrios, 1997). Plant pathogens are known to produce and use enzymes in the penetration of cell walls. Cell wall-degrading enzymes play important roles, but the activity of these enzymes is hardly detectable in numerous obligate parasites such as *U. viciae-fabae* (Cooper, 1984). More recently, Heiler, Mendgen and Deising, (1993) confirmed the presence of cellulase activity in *U. viciae-fabae*, and the production of this enzyme is neither substrate-inducible nor catabolite repressible. In addition, these cellulotic enzymes of *U. viciae-fabae* are formed during the differentiation of infection structures. This is contrary to cellulases of necrotrophs and saprophytes, which are non-differentiation specific (Heiler *et al.*, 1993).

The other enzyme reported to have been used by *U. viciae-fabae* to penetrate its host cells is pectinesterase. This enzyme alters the chemical composition of pectic substances (into demethylated pectin) at the site of infection (Frittrang *et al.*, 1992). Polygalacturonate lyase further attacks the latter and cleaves the pectic chain (Deising and Mendgen, 1992; Frittang *et al.*, 1992).

More recent evidence suggests that *U. viciae-fabae* can produce extracelluar proteinases capable of breaching the host cell wall by degrading fibrous, hydroxproline-rich proteins. The latter are important in plants for cell wall stability and play a role in defense against fungal pathogens (Rauscher, Mendgen and Deising, 1995). Together with cellulase, proteinase weaken cell walls and facilitate infection and the intracellular invasion of host tissues by *U. viciae-fabae*.

After a rust fungus enters its host cells, it establishes contact with the cells and procures (absorbs) nutrients. This event is referred to as infection. Symptoms or signs (appearance of uredosori or pustules) are expressions of successful infection (Zadoks and Schein, 1979; Agrios, 1997). The length of time between inoculation and appearance of visible pustules (latent period) is dependent on factors related to the pathogen, development stage and genotype of the host plant (Parlevliet, 1975). For example, Sache and Zadoks (1995a) recorded a latent period of 8 days for the rust, *U. viciae-fabae*, on a susceptible faba bean cultivar, Alfred.

Richmond (1983) showed that *U. viciae-fabae* is capable of spreading into a cell by growing directly through the cell (intracellular) and/or by growing between cells (intercellular). After tissue colonization, rust fungi eventually reproduce in their infected host. *U. viciae fabae* reproduces by means of spores, urediniospores (asexual) and teliospores that are capable of producing basidiospores (sexual spores) upon germination (Prasada and Verma, 1948; see also section on biology). Once a parasitic fungus such as a rust has consumed the colonized tissue, it must move to the next feeding site or susceptible host in order to survive (Zadoks and Schein, 1979). To accomplish this task, it must disperse (Zadoks and Schein, 1979). In order to be dispersed, dispersal units (spores) have to be carried by some kind of agent. In this regard, wind plays an important role in disseminating rust fungi. A question related to the movement of fungal pathogens is at what speed do they travel and what mechanisms of spore dispersal are operational. Field studies suggest that the faba bean rust, *U. viciae-fabae*, spreads at a wave velocity of ca. 0.1 m per day (Sache and Zadoks, 1996; Negussie, 1991). The observation that distance parameters varied with respect to source and trap plots suggests that two

mechanisms of spore dispersal, namely a short-distance, high frequency, deterministic mechanism and a long-distance, low frequency stochastic mechanism are involved in dispersal of *U. viciae-fabae* (Sache and Zadoks, 1996).

If a rust pathogen does not obtain a susceptible host for various reasons then it will have to use an alternative means of survival (Zadoks and Schein, 1979). *U. viciae-fabae*, for example, survives the unavailability of host plants as teliospores in infected plant debris in the soil or mixed with seeds (Prasada and Verma, 1948).

PHYSIOLOGICAL AND ANATOMICAL CHANGES OF PLANTS INFECTED WITH *UROMYCES VICIAE-FABAE*

Pathological changes in diseased plants are the result of changes in the structure, organization (anatomy), and functions (physiology) of the cells and tissues affected (Šutic and Sinclair, 1991). Rust infections are thought to affect water uptake and transport as a result of altered anatomy of root tissues of rusted plants (Paul and Ayres, 1986). For example, rust infection of faba bean by *U. viciae-fabae* reduced root diameter, increased specific root length (cm root mg^{-1}), and inhibited length increases of tap and primary lateral roots in soil columns (Tissera and Ayres, 1988). Attack by *U. viciae-fabae* also inhibits the growth of roots in the mid-depth region of soil columns and this will undoubtedly reduce water uptake in rusted plants during a period of drought stress (Tissera and Ayres, 1986). This may have important implications for lentils in low moisture stress areas since there are shallow-rooted types with root length of about 15 cm (Saxena and Hawtin, 1981).

Rust infection also affects the production of cellular substances. Buonaurio, Passeri and Torre (1989) have shown a decrease in lipids in leaves infected by *U. viciae-fabae*, Moreover *U. viciae-fabae* infection significantly reduces the chlorophyll a/b ratio of chloroplasts (Buonaurio, 1991), and these substances have a vital role to play in photosynthesis. Clearly, the reduction in lipids and chlorophyll a/b ratio brings about the reduction in the rate of photosynthesis, which in turn results in yield reduction of rusted plants.

DISEASE MANAGEMENT

Strategies being used in many countries to manage lentil rust include fungicide application, manipulation of planting date, destruction of infected host debris (cultural measures), and the use of resistant cultivars.

Chemical measures

There are several fungicides which are effective against lentil rust when used as foliar sprays and seed-dressings. For example, Mohyud-Din, Khan and Khan (1999) found mancozeb effective when applied either as a foliar spray or as a seed treatment. Mancozeb, propiconazole, triadimefon, oxycarboxin, thiram, tebuconazole tridemorph, benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and metiram are commonly used fungicides to control rust diseases of food legumes caused by *Uromyces* spp. and lentil rust in particular (Yeoman *et al.,* 1987; Rashid and Bernier, 1991; Marcellos, Moore and Nikandrow, 1995; Pande, Srivastava and Shahi, 1995; Ayub *et al.*; 1996; Fontem and Bouda, 1998; Habtu, Zadoks and Abiye, 1998; Mohuyd- Din *et al.*,

1999). Singh (1985) reported the efficacy of carbendazim and triadimefon against lentil rust, and suggested that the fungicide delays the onset of the disease when applied as seed treatment. Al-Zumari (1994) reported the effectiveness of procymidone, benomyl, cymoxanil + mancozeb, metalaxyl + mancozeb and oxadixyl + folpet against rust, *U. fabae*, in faba bean.

Spraying lentil crops with a mixture of triadimefon (0.5 kg ha^{-1}) and propinet (2 kg ha^{-1}) at pre-flowering, full flowering and early pod maturity stages has been reported to be effective in controlling rust and thereby increasing seed size and yield (Sepulveda and Alvarez, 1989). In Egypt, the efficacy of benomyl, carboxin, metalaxyl, oxycarboxin, thiram, triadimefon and triforine spray either singly or in combination with mancozeb against faba bean rust has been proven (Khaled, El-Moity and Omar, 1995). In India, Sugha, Chauhan and Singh (1994) reported the effectiveness of thiabendazole, benomyl, carbendazim, thiophanate-methyl, triadimefon, dinobuton and myclobutanil against the pea rust fungus, *U. viciae-fabae*. Although in their experimental stage, synthetic putrescine analogues, (E)-1,4-diaminobut-2-ene (E-BED) and (E)-(N,N,N',N'-tetraethyl)-1,4-diaminobut-2-ene (E-TED) were shown to be effective in controlling the rust disease through their effect in the reduction of germination and appressorium formation by urediniospores of *U. viciae-fabae,* and E-BED (Reitz *et al.*, 1995).

The effectiveness of each of the above fungicides is dependent upon time and frequency of application. Some fungicides are effective at early stages of rust infection and others at a later stage since there are differences in sensitivity between aeciospores and urediniospores to specific fungicides (Sugha *et al.*, 1994). For example, aeciospores are most sensitive to thiabendazole, followed by benomyl, carbendazim, and thiophanate-

methyl, and least sensitive to bitertanol, whereas urediniospores are most sensitive to triadimefon, dinobuton and myclobutanil and least sensitive to bitertanol (Sugha *et al.*, 1994).

Applying a fungicide twice after rust appearance is more effective than applying once before onset of the disease (Mohyud-Din *et al.*, 1999). The beneficial effect of repeated applications of mancozeb, when *U. viciae-fabae* appears early in the season and becomes severe, has been reported in faba bean by Yeoman *et al.* (1987).

Similar to the frequency of application, the time interval between successive sprays is also influential on the efficacy of a fungicide. The interval could be large or small depending up on the progress of the rust epidemic. For example, Yeoman *et al.* (1987) applied fungicides twice at an interval of ca. 42 days and managed to effectively control rust caused by *U.viciae-fabae*. Had there been rapid progress of the rust epidemic, they would have used shorter intervals and more than two sprays.

There is an obvious reduction in yield from rusted lentil crops. However, there is no explicit account of how the disease affects the yield of the crop. In other food legume crops such as faba bean, the yield increase from rust control is due to an increase in one of the yield components, i.e. 100-seed weight (Yeoman *et al.*, 1987; Rashid and Bernier, 1991; Marcellos *et al.*, 1995). Similarly, Sache and Zadoks (1995a) reported that *U. viciae fabae* infection reduces yield of faba bean by decreasing the yield components, namely seed weight/stem, seed weight and the number of pods/stem.

When applied, some fungicides appear to prolong the growth of a crop or delay leaf senescence. This is what Zadoks and Schein (1979) referred to as a “tonic effect”. Such an effect has been reported for mancozeb and tebuconazole when applied to control

rust in faba bean (Marcellos *et al.*, 1995) and propiconazole when applied to control rust in wheat (Negussie personal observation, 1996). In general, fungicides often designated as azoles are known to have growth regulatory effects on both monocotyledonous and dicotyledonous plants, and the typical effects reported include increased resistance against different kinds of stress and delayed senescence (Kuck, Scheinpflug and Pontenzen, 1995).

In general, fungicides are not used for controlling lentil rust in developing countries like Ethiopia for these reasons: (1) many are expensive and unavailable to lentil growers, (2) almost all require technical know-how that farmers lack, and (3) better alternatives such as resistant varieties are available.

Cultural measures

Crop health is affected by cultural practices such as planting time, plant density (seeding rate) cropping sequence, cropping pattern, fertilization, seed-bed preparation, weeding, etc. Such practices can also influence the level of biotic stresses and their effect on the growth and yield of a crop. For example, delaying sowing date reduced lentil rust severity in Ethiopia (DZARC, 1992; Mengistu and Negussie, 1994). However, in spite of the high rust infection, early sown lentils gave significantly higher seed yield than the late-sown ones. In India, delayed sowing of lentil effectively reduced rust disease, and the disease incidence was less in lentil sown as a mixed crop with wheat than as a sole crop (Mittal, 1997).

Environment, in general, has an influence in plant disease development, and this also applies to lentil rust. Variation in reaction to rust due to location and genotype by

location interaction has been reported by Vir and Gupta (1994). A similar phenomenon occurs in Ethiopia (Negussie, unpublished observation). Therefore, use of selected lentil production areas, depending on the variety, may reduce the rust problem.

Physical measures

As was referred to above (see section on biology), rust infected plant debris left in the field and carried with lentil seeds give rise to fresh infection of the crop in the succeeding year. Thus, burning rust infected lentil plant debris to keep fields free from teliospores and cleaning lentil seed to remove residues help in minimizing rust occurrence the following season (Prasada and Verma, 1948). The destruction of infected plant debris would significantly reduce the survival of primary inocula, i.e. teliospores of *U. viciae-fabae*, and reduce the loss due to severe epidemics.

Escape

Lentil cultivars have been developed that mature before development of severe rust epidemics. For example, the early maturing cultivar "Checkol" (NEL-2704) in Ethiopia (Bejiga, Million and Yadeta, 1996) shortens the number of generation times available for the development of lentil rust epidemics, and allows it to escape serious damage. Encouraging the early maturing trait in a genotype, without sacrificing yield, might thus be advantageous to the lentil producer.

Induced resistance

Practical and successful control of plant diseases through induced resistance has been reported for several crops (Kuc, 1982; Kuc, 1987). This control strategy is based on a mechanism referred to as induced systemic resistance (ISR) (Kuc, 1987). Systemic resistance can be induced either by disease causing agents (Kuc, 1987) or chemical means (Walters and Murray, 1992). Inoculation of lower leaves of *Vicia faba* with *U. viciae-fabae*, has been demonstrated to increase resistance to rust infection in the upper uninfected leaves, i.e. ISR (Murray and Walters, 1992). The systemic resistance induced by *U. viciae-fabae* in unaffected upper leaves of rusted plants is explained by the increased rates of photosynthesis, which is thought to facilitate the expression of resistance (Murray and Walters, 1992)

Systemic resistance to *U. viciae fabae* infection can also be induced by potassium phosphate or ethylene diaminetetra-acetic acid (EDTA) (Walters and Murray, 1992). Induced resistance is a potentially promising rust management approach, and might be of use in protecting lentil plants against *U. viciae-fabae*. Lentil breeders may give due consideration to this approach since the ability to induce resistance in susceptible plants implies that the genetic potential for rust resistance is in all plants (Kuc, 1982).

Host plant resistance

Rust resistant varieties and genetics of rust resistance

Use of host plant resistance to manage diseases is economical, long-lasting, effective, easy to handle and environment-friendly. Cultivation of high yielding lentil varieties possessing rust resistance is becoming customary in the farming systems of most lentil

growing countries, developing counties in particular, where rust is a key problem of production. Numerous resistant varieties are available in countries of Africa, South America and South Asia (Bayaa and Erskine, 1998). In Bangladesh, for example, 'Barimasur-4' (derived from a cross ILL 5888/FLIP 84-112L) was released in 1995 (Sarker *et al.*, 1999). Similarly, 'Pant Lentil 4' was released for commercial cultivation in the northwestern plains of India (Singh *et al.*, 1994). Alemaya (FLIP-89-63L) has been recently approved for release in Ethiopia (Bejiga, Negussie and Erskine, 1998).

The success rate of developing improved lentil cultivars with resistance to rust is relatively high because of simple mendelian inheritance of the trait (Sinha and Yadav, 1989; Singh and Singh, 1990; Singh and Singh, 1992). In addition, there are indications that resistance could also be controlled by duplicate dominant genes in macrosperma lentils e.g. the variety Precoz (Chuhan, Singh and Singh, 1996; Chuni *et al.*, 1996; Kumar, Singh and Singh, 1997). The International Center for Agricultural Research in the Dry Areas (ICARDA) contributes significantly to the development of resistant varieties by coordinating and distributing screening nurseries to national lentil programs in countries where rust is a problem such as Ethiopia, Morocco and Pakistan (Erskine *et al.*, 1994a).

In faba bean (*Vicia faba*), variable degrees of resistance to specific races of *U. viciae-fabae* have been reported (*Vicia faba*) (Rashid and Bernier, 1984). Some of the resistance genes are known to provide resistance either to a single race or to more than one race of *U. viciae-fabae* (Rashid and Bernier, 1986; Rashid and Bernier 1984; Conner and Bernier, 1982c). This suggests there could be more than one gene in lentil conditioning resistance to certain isolates or combination of isolates of *U. viciae-fabae*.

Testing for rust resistance

Any disease screening procedure or technique should provide heavy disease pressure, be efficient, reliable, practical and cost-effective. Accordingly, screening genotypes for their resistance to rust in hot-spot areas under field conditions where the environment for lentil growth and rust development is favourable, is a common practice worldwide. Figure 1.5 shows field screening for rust resistance at Akaki, Ethiopia.

Although there is no evidence of the break down of resistance to lentil rust, pathogenic variability within the lentil rust population has been reported by Singh *et al.* (1995). This suggests the need for screening lentil genotypes against rust under controlled environments as a supplement to field screening. One problem in controlled environments is space limitation so that a large population cannot be evaluated at one time. The other problem is unavailability of facilities for controlled environments, especially in developing countries.

Despite the above-mentioned disadvantages of controlled environments, a few advanced materials can be effectively screened. Outlines of glasshouse and laboratory methods to inoculate rust have been given by Kramm and Tay (1984) and Singh and Sokhi (1980), respectively.

In the course of inoculation, the density of spores influences germination and subsequent infection. According to Kramm and Tay (1984), a concentration of 3×10^4 urediniospores of *U. viciae-fabae*/ml is optimal for infection. However, germination of urediniospores of *U. viciae-fabae* is reduced in proportion to the increase in spore concentration as the concentration is increased above 2×10^4 urediniospores/ml due to self inhibitors (Parker and Blakeman 1984b). Therefore, this indicates that optimization

of urediniospore concentration requires refinement to obtain a level of infection by *U. viciae-fabae* better than what is achieved with the inoculum concentration currently suggested for controlled environment studies.

Sources and identification of resistance

Sources of resistance to rust are present in the cultivated species of *Lens* itself, i.e. *Lens culinaris*. Previously, numerous *L. culinaris* breeding lines or germplasm accessions from different lentil growing countries have been identified as resistant to rust. Published sources have been reviewed by Porta-Puglia *et al.* (1994), but additional/new sources have been identified since their review. These additional sources of resistance or lentil germplasm accessions or breeding lines are listed in Table 1.4.

In principle, accuracy (closeness of a sample estimate), precision (repeatability or variation associated with a sample estimate), reproducibility (lack of variation in estimate when the same disease sample is re-evaluated by another evaluator) and efficiency are considered essential attributes of a successful disease assessment scheme (Campbell and Madden, 1990). In order to screen and identify a large number of lentil entries under field conditions, use of a disease rating scale that gives results, which are reasonably accurate, efficient and reproducible, will have to be used. The term disease rating scale, here, is used to describe in words or numbers, the disease classes (usually ranging from no disease to fully diseased) observed for plant parts, whole plants, plots, or fields (Nutter, Teng and Shokes, 1991).

In line with the above-mentioned facts, many lentil breeders and pathologists use a 9-point rating scale based on visual judgement of disease severity. Although small

variations exist, the scoring scale was standardized by ICARDA. This standard scale together with the variants is given in Tables 1.5-1.9.

A good quality disease scale should help differentiate among the test materials by providing adequate resolution of differences in disease severities (discriminating capability), and this can be achieved by having sufficient classes in the scale (Campbell and Madden, 1990). Some scales have difficulties in maintaining this quality. The rating scales given in Tables 1.5 and 1.6, for example, have reasonable discriminative capability or resolving power; however, a scale listed in Table 1.9 appears to have a large range value for each class, if the scale divides the range of disease intensity into equal classes. Likewise, the disease classes in the scale used by Singh and Sandhu (1988), as indicated in Table 1.8, are not 50% symmetrical. Hence use of such rating scales under conditions of low rust intensity, or when there is high disease pressure, may not be appropriate.

In addition to the difficulties mentioned above, some of the scales combine yield with symptoms. An example is given in Table 1.6 in which Singh and Kant (1999) used the term "tolerant" to represent average host reaction or a moderately susceptible reaction type. It will be conceptually misleading however if the term is interpreted in the strict sense, especially for fungal diseases. It is difficult, if not impossible, to identify and evaluate tolerance with such a disease evaluation technique. It is important to consider the evidence for tolerance. Schafer (1971) defined tolerance as "that capacity of a cultivar resulting in less yield loss relative to disease severity or pathogen development when compared with other cultivars or crops." This was exemplified by the same yield loss (20%) for both non-tolerant and tolerant varieties with 40% and 80% disease severity, respectively.

The method of disease assessment mentioned earlier is not satisfactory for evaluating materials in the greenhouse as in such experiments it would be necessary to have precise measurements of the reaction of the test materials to the disease. Such a scale should combine qualitative as well as quantitative measurements in order to be of use in epidemiological studies. In addition, an assessment key based on percentage leaf (plant tissue) area covered with rust need to be used to estimate the plant area infected. However, the standard scale remains to be a powerful tool to measure cultivar reaction in the field. The results of such measurements are helpful to study the relationship between field reaction and components of resistance in the glasshouse and determine whether it is worth considering the components as criteria of selecting for rust resistance (Savary *et al.*, 1995).

Mechanisms of rust resistance in lentil

In general, genetically controlled morphological/structural and physiological characters or factors are responsible for disease resistance in plants (Chaudhari, 1986). In their review, Porta-Puglia *et al.* (2000) emphasized the contribution of morphological and physiological mechanisms of resistance to breeding for disease resistance in food legume crops. Considering morphological mechanisms, Reddy and Khare (1984) reported that resistance of lentil varieties to rust is due to the increased quantities of wax on their leaf surfaces.

In addition, Reddy and Khare (1984) reported physiological differences between resistant and susceptible lentil varieties in the absence of rust. Accordingly, the quantities of reducing sugars are greater in leaves and seeds of susceptible cultivars than in resistant

cultivars. Potassium, phosphorus, calcium, magnesium, iron and manganese levels were higher in leaves, stems and seeds of resistant varieties. The quantities of nitrogen and zinc, however, were higher in leaves and seeds of susceptible varieties. Relating simple elements and biochemicals to rust resistance would be presumptions. Further research is necessary before definite conclusions can be drawn on the biochemical compounds related to rust resistance in lentils. Investigations by Mohase (2003), for instance, have shown that attack by rust (*Puccinia helianthi*) induces the production of pathogenesis-related (PR) proteins (β-1,3-glucanase, chitinase and peroxidase) which are involved in reducing disease development in the resistant sunflower line PhRR3. Salycilic acid has also been shown to be the compound responsible for the induction of PR proteins (Mohase, 2003).

Evaluation of lentil germplasm collections originating from 18 countries revealed that 30% and 37.5% of the landrace accessions from India and Ethiopia, respectively, exhibited symptoms of iron (Fe) deficiency (Erskine, 1997). Interestingly, the majority of Ethiopian landraces are susceptible to rust (Negussie, personal observation). It would, however, be speculative to associate low iron content with susceptibility. Further investigation needs to be done to clearly establish the relationship between this physiological phenomenon and disease reaction.

Current evidence suggests that resistance or susceptibility to rust is a function of morphological and/or physiological traits of lentil varieties. The presence of chemical constituents such as nitrogen and reducing sugars in certain lentil varieties favour the establishment of the rust pathogen making them less resistant to the rust. However, it is not clear whether or not these substances have a primary role in determining resistance or

susceptibility to rust in lentils. Perhaps there may be other mechanisms having a decisive role in lentil's reaction to the rust. For example, high concentrations of sugars and amino acids contained in leachates (extracellular substances) of faba bean leaves inhibit urediniospore germination, growth of germ tubes and infection by *U. viciae-fabae* (Parker and Blakeman, 1984a). Future research on resistance mechanisms to lentil rust will help to answer this question.

CONCLUSIONS

There is no standard set of lentil rust differentials that can be used for race identification. Therefore, a set of differentials needs to be developed and standardized. Moreover, molecular techniques should also be exploited to identify pathogenic variation in *U. viciae-fabae*. Such techniques are widely used to characterize many pathogens (Maclean *et al.*, 1995; Sandlin *et al.*, 1999; Dobinson *et al.*, 2000).

The disease-loss information available in the literature cannot readily be used to justify the use of any control measures. To justify the use of resistant cultivars or any other method, it is not sufficient to mention yield loss alone. The magnitude of the loss must be related to the amount of disease and, of course, to the gain obtained. This can be achieved through disease-loss appraisal (James, 1974). Therefore, field experiments need to be conducted to characterize the relationship between rust and loss in lentil yield, so that a reliable method can be developed to estimate the loss in lentil yield associated with any given amount of rust disease.

It is known that rust resistance in lentil is monogenic or oligogenic. Although monogenic and oligogenic resistance is not proof of vertical resistance, vertical resistance

is often inherited as discrete genes. Vertical resistance usually is a temporary type of resistance leading to a boom-and-bust cycle of production in which case there will be a quick resistance breakdown. In Kenya, for instance, the average commercial life of a wheat cultivar with vertical resistance was estimated at 4.4 years (Robinson, 1976).

The above phenomenon occurs due to the positive selection pressure exerted on the pathogen or the imposition of physiological change in the pathogen population. The greater the extent of production, both in time and space, the greater the selection pressure. Moreover, in countries like Ethiopia where rust is a major constraint to lentil production and the cultivated area is exclusively sown with landraces (Solh and Erskine, 1981), the replacement of these landraces with rust resistant and improved cultivars will cause genetic erosion. Therefore, breeding lentil for partial resistance, a type of horizontal resistance, to *U. viciae-fabae* would seem likely to minimize the danger of reducing the effective life span of rust resistant lentil cultivars.

No evidence is available on the growth stage of the lentil host at which rust resistance is expressed. In other words, it is not clear whether resistance is expressed in mature plants, in seedlings or in both. Lentil breeding programmes usually select those plants that show resistance at maturity. Hence, studies on lentil rust considering the different growth stage need to be done to clarify this point.

Information on such epidemiological aspects as the relationship between inoculum density and disease development is lacking. Apparently, pathological studies on such epidemiological aspects are imperative to generate the information necessary to establish the relationship between the two.

The benefits of combined use of the different lentil rust management strategies are worth investigating in order to developing an integrated lentil rust management programme.

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Table 1.1 Lentil production and yield of major lentil growing countries during 2003 (Source: FAO, 2004)

Country*	Lentil area (1000 ha)	Lentil production (1000 MT)	Lentil yield (kg ha^{-1})
India	1344	833	620
Canada	536	520	971
Turkey	500	548	1096
Iran	270	105	389
Nepal	182	148	813
Bangladesh	154	116	752
Syria	139	168	1231
Australia	128	207	1617
USA	96	111	1155
China	90	132	1467
Ethiopia	75	38	510
Morocco	54	34	620
Pakistan	46	29	638
Spain	29	21	748
Russian Federation	15	12	798
Africa	141	81	577

* Countries with lentil area of ≥ 10, 000 ha.

Table 1.2 Host range of *Uromyces viciae-fabae*

Host	Country	Source
Lathyrus arizonicus, L. bijugatus, L. bolanderi, L. coriaceus, L. decaphyllus, L. laetivirens, L. leucanthus, L. littoralis, L. myrtifolius, L. muttallii, L. obovatus, L. ochroleucus, L. oregonensis, L. palustris, L. pauciflorus, L. polyphyllus, L. stipulaceus, L. strictus, L. sulphurous, L. torreyi, L. utahensis, L. venosus, L. violaceus, L. watsonii, Vicia americana, V. angustifolia, V. californica, V. cracca, V. disstifolia, V. faba, V. melilotoides, V. oregana, V. sativa, V. sparsifolia		Arthur (1962)
Lens, Lathyrus, Pisum and Vicia		Laundon and Waterson (1965)
Lens culinaris, Vicia faba	Ethiopia	Stewart and Dagnachew (1967)
Lathyrus aphaca, L.sphaericus,Pisum abyssinicus,P.elatus, P. jomardi,Vicia biensis, V.ervilia,V.gracilus,V. hirsuta,V.narborensis, V. tetrasperma	India	Kapooria and Sinha (1971)
Lathyrus japonicus, L. latifolius, L.ocroleucus, L. odoratus,L.sativus, L. tuberosus,L.venosus, Lens culinaris, Pisum sativum,Vicia americana,V. calcarata, V. cracca, V. disperma, V. ervilia, V. faba, V. monatha	Canada	Conner and Bernier (1982b)
Lens culinaris	Algeria, Argentina, Chile, Cyprus, Egypt, Iran, Pakistan, Italy, Morocco, Portugal, Nepal, Syria, Turkey and	Beniwal *et al*. (1993)
Vigna radiata	India	Madhu *et al*. (1998)
Vicia faba	India, Yugoslavia, China, Egypt, Australia, England, Syria	

Table 1.3 Races reported in *Uromyces viciae-fabae*

Country	Number of races	References
Former Yugoslavia	9	Kispatic (1950)
India	6	Singh and Sokhi (1980)
Canada	11	Conner and Bernier (1982a)
India	5	Singh *et al.* (1995)

Table 1.4 Lentil breeding lines or germplasm accessions resistant to rust

India		Ethiopia	
Line	Line	Line	Line
L-1335	P212	FLIP 84-54L	LL 57
L-1336	P213	FLIP 86-38L	76TA-66116
L-1569	P467	74TA-441	Pant L-406
L-1570	P468	FLIP 86-16L	ACC-36152
L-2522	ILL 18	FLIP 84-112L	PGRC-7
L-2586	PL 638	FLIP 89-51L	
L-2929A	NEL 115A	FLIP 84-75L	
L-2948	NEL 149A	FLIP 86-83L	
L-2950	NEL151	FLIP 86-42L	
L-2951	NEL 153	FLIP 87-21L	
L-3050	NEL 277	FLIP 84-2L	
L-3107	NEL 350	FLIP 85-33L	
L-3115	NEL 362	FLIP 86-22L	
L-3899	NEL 182	FLIP 87-52L	
L-3965	NEL 1056	FLIP 88-47L	
L-40006	Syrian local	FLIP 89-30L	
L-4084	ILL 4605	ILL 4065	

References:
Ethiopia: Bejiga *et al.* (1995); Bejiga and Yadeta (1999); Negussie (Personal observation, 1997).
India: Kumar *et al*. (1997); Singh and Kant (1999).

Table 1.5 A 9-point standard lentil rust rating scale established at ICARDA, Syria

Grade	Typological description	Reaction
1	No pustules visible	Resistant
3	Few scattered pustules, usually seen after careful searching	Moderately resistant
5	Pustules common on leaves and easily observed, but causing no apparent damage	Average reaction
7	Pustules very common and damaging but not observed on petioles and stems	Moderately susceptible
9	Pustules extensive on leaves, petioles and stems, and killing leaves and other plants	Highly susceptible

Table 1.6 A 9-point scale used to characterize reaction of lentil lines/germplasm to rust at Pantnagar, India (Singh and Kant, 1999)

Grade	Reaction
1	Free from infection
3	Resistant
5	Tolerant
7	Susceptible
9	Highly susceptible

Table 1.7 A 1-9 rating scale that takes aecial cups into account and used to evaluate resistance to rust in lentil (Khare *et al.*, 1993)

Grade	Description
1	1-10% leaves have no more than two aecial cups or uredosori
3	1-10% leaves have no more than five aecial cups or uredosori
5	11-25% leaves affected
7	25-50% leaves affected
9	Over 50% leaves affected

Table 1.8 A 9-point scale used to characterize reaction of lentil lines/germplasm to rust in Punjab, India (Singh and Sandhu, 1988)

Grade	Disease intensity	Reaction
1	No infection	Resistant
3	Up to 10% leaf area infected	Moderately resistant
5	10.1-25% leaf area infected	Moderately susceptible
7	25.1-50% leaf area with stem also infected	Susceptible
9	Above 50% leaf area with stem, as well as pods, heavily infected	Highly susceptible

Table 1.9 A 5-point scale used to characterize reaction of lentil lines/ germplasm to rust in Chile (Bascur and Sepulveda, 1989)

Grade	Description
0	No symptom
1	Few uredosores found only on the basal leaves of the plant
2	Uredosores present on leaves throughout the plant
3	Abundance of uredosores on stems and leaves with mild defoliation
4	Uredosores on leaves, stems, petioles and pods with severe defoliation
5	Severe defoliation and death of the plants

Figure 1.1 Rust symptoms on lentil: (A) orange yellow-coloured aecia on the leaves, (B) aecia and brown-coloured uredinia on leaves and stems, (C) aecial cups borne in a ring-like cluster on the upper surface of a leaflet, (D) aecial cups on the lower surface of a leaflet (E) close-up of aecial cups on a leaf surface (F) uredinia on leaves and stems, (G) uredinia on leaves and stems two weeks after inoculation, (H) uredinia on leaves and stems three weeks after inoculation, and (I) telia mostly on stems and petioles.

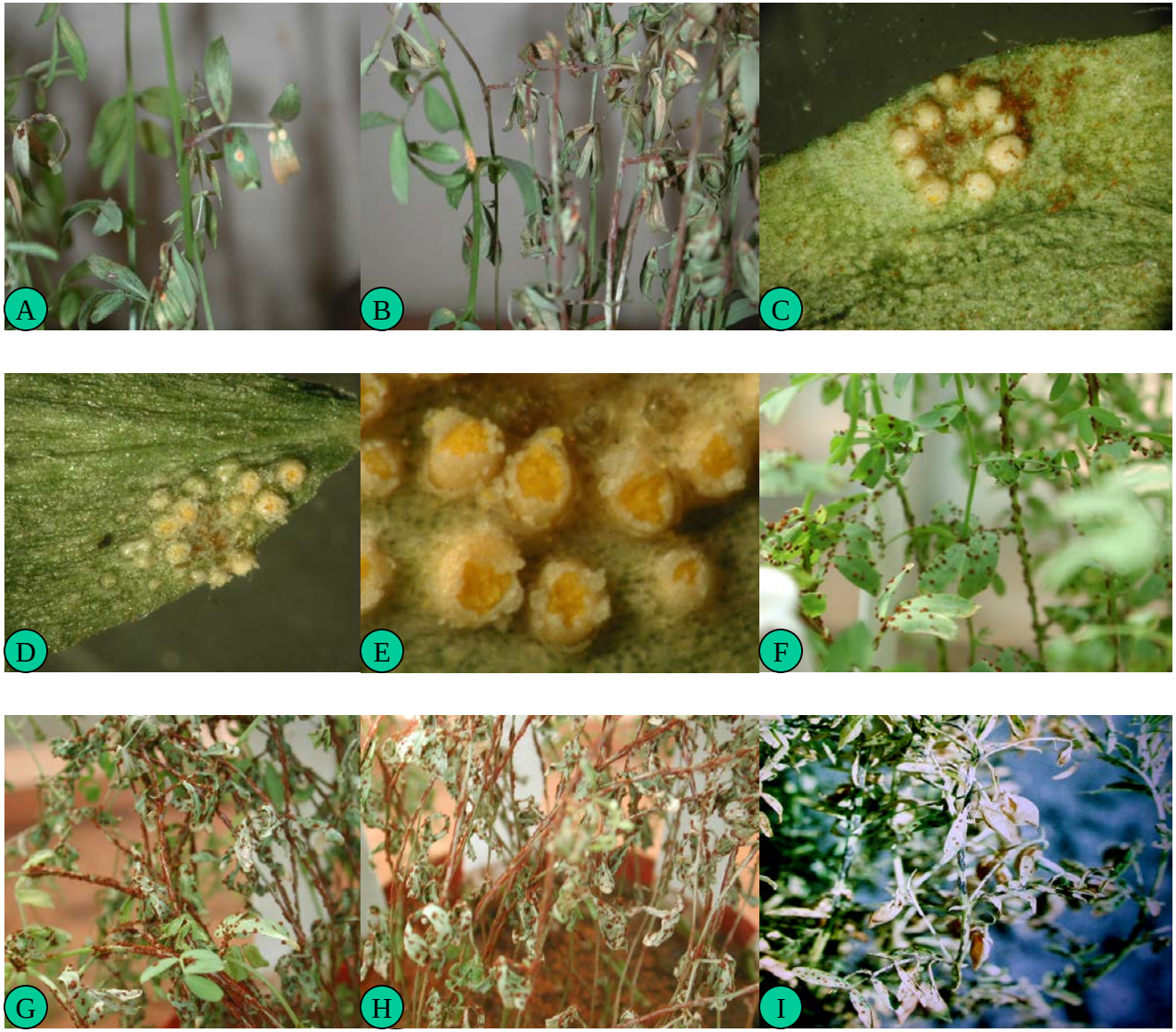
A
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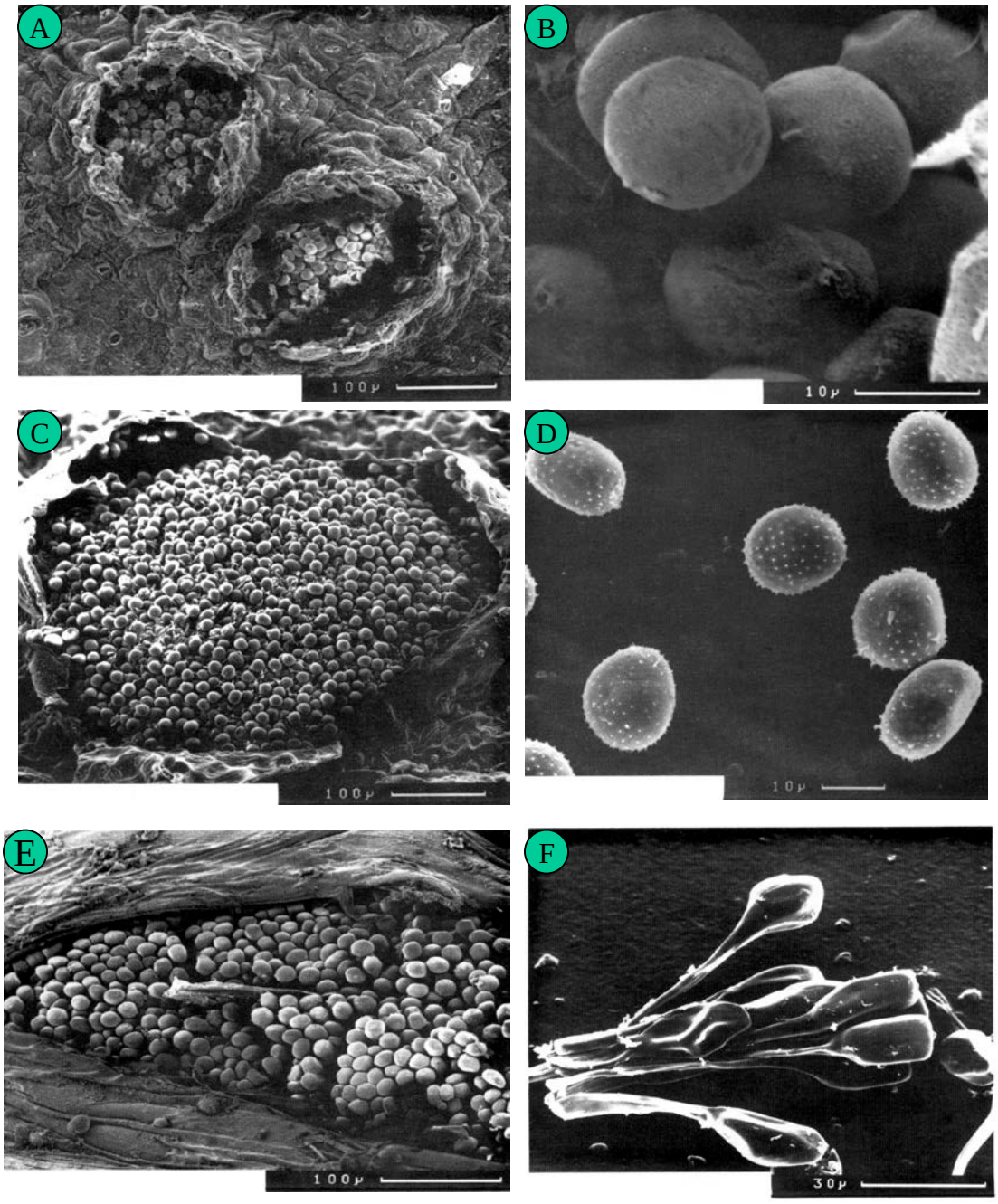
Figure 1.2 Lentil cultivars in a farmer's field at Chefe Donsa, Ethiopia: rust-free healthy cultivar Adaa (left) and rust-devastated local cultivar (right).

Figure 1.3 Scanning electron micrographs of spore bearing structures and spores of *Uromyces viciae-fabae*. (A) aecium; (B) aeciospores; (C) uredinium; (D) urediniospores; (E) telium; and (F) teliospores (Atlas species 155 from Preece and Hick, 1990).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D
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E
100μ
F
30μ

Figure 1.4 Geographical distribution of *Uromyces viciae-fabae* (based on CMI distribution map of plant diseases, 1990 (reproduced from the Crop Protection Compendium, Global Module, 2nd Edition. ©CAB International, Wallingford, UK, 2000).

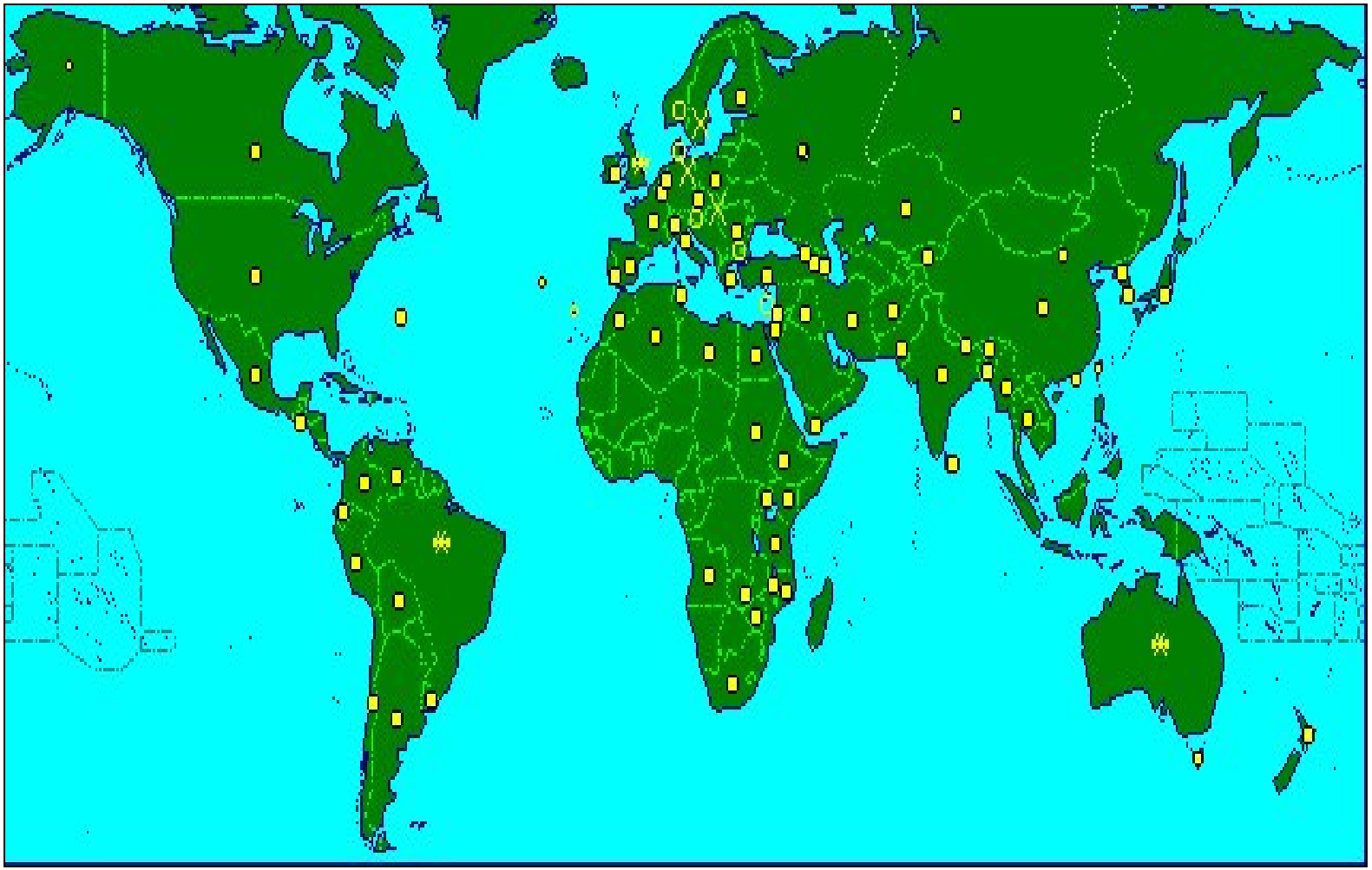

Figure 1.5 Field screening for lentil rust at the Akaki research station of the Debre Zeit Agricultural Research Center, Ethiopia. Brown lines are rusted (susceptible) and green/greenish yellow are resistant.

CHAPTER 2

SEQUENTIAL ANALYSES OF LENTIL RUST EPIDEMICS

"No one can be a good observer unless he is a good theorizer."

Charles Darwin, as quoted by J.C. Zadoks

"In many aspects integrated pest management (IPM) represents the basis for problem-solving in pest management, leading ultimately to increased benefits to the farmer, the consumer and society as a whole. There is, however, another aspect of pest management- - the problem definition aspect. This problem definition aspect of pest management contains a collection of methods which has come to be known as CROP LOSS ASSESSMENT."

P.S. Teng

ABSTRACT

Sequential (cross-sectional and longitudinal) analyses of rust (*Uromyces viciae-fabae*), lentil growth, yield and yield components were performed by assessing crop and disease parameters at different crop growth stages and leaf canopy layers in five field rust epidemics at Akaki, Ethiopia. The epidemics were created by artificial inoculation of the rust-susceptible cultivar EL-142 with an Akaki isolate of the rust fungus and spraying of tebuconazole at various frequencies. The five epidemics produced similar-shaped disease progress curves that varied significantly in the rate of temporal progression (r_L) and area under the disease progress curve (AUDPC). The epidemics, however, did not significantly affect crop growth. The rust epidemic had a significant influence on seed yield, pods per plant and seed mass between early flowering and early pod formation. The number of seeds per pod remained unaffected by the disease. The rust also significantly affected harvest index and days to maturity. Results demonstrated that a seed yield loss of up to 41.7% could occur and the effect of rust on seed yield of lentil can be predicted with AUDPC and critical-point models using disease severity assessed on the upper canopy layer in the early flowering stage. Every 1% increase in rust severity reduced seed yield by 8.39%. Rust severity ≥ 4.7% at the critical stage, early flowering, will significantly reduce seed yield, and, hence, any measure that keeps rust severity below this threshold level would be advisable.

INTRODUCTION

Rust (*Uromyces viciae-fabae*) is the most important disease of lentil (*Lens culinaris* Medik.) in Africa (Ethiopia and Morocco), Latin America (Chile and Ecuador) and South Asia (Bangladesh, India and Pakistan) (Erskine and Saxena, 1993). It can cause serious yield losses in many countries, in some cases as high as 100% (Khare, 1981; Bejiga, Negussie and Erskine, 1998). However, most of these loss estimates are based on expert guesses and no quantitative data are available that show the effect of rust on lentil growth, yield and yield components.

Quantitative data on the relationship between disease and crop parameters are useful for the understanding of rust epidemics and for crop loss assessment and rust management. Such data can be generated through sequential analysis, a quantitative analysis of a sequence of events by means of a combination of cross-sectional and longitudinal studies (Zadoks, 1972; Zadoks, 1978). This analysis has been effectively used in establishing relationships between diseases, crop growth, yield and yield components. For example, Habtu, Abiye and Zadoks (1997) and Habtu and Zadoks (1995) used this approach in the bean-rust pathosystem in Ethiopia. In lentil, however, no such analyses or studies have been made. Development of disease-yield loss relationships require studying the disease throughout the season as well as monitoring growth of healthy and diseased plant populations, i.e. disease-yield loss appraisal (James, 1974).

The objectives of the study were, therefore: (1) to determine the effect of different rust epidemics on leaf area index, yield and yield components; (2) to establish quantitative relationships between rust and crop parameters and between different crop parameters, (3) to characterize descriptors and temporal progression of lentil rust

epidemics, and to generate information that can aid in assessing yield losses in lentil due to rust.

MATERIALS AND METHODS

The experiment was carried out on light black vertisol at Akaki Research Station (38º 53' 11" E; 8º 53' 21" N; 2200 m.a.s.l. and *ca*. 1000 mm annual rainfall), Ethiopia during the 2001/2002 cropping season. Weather parameters (rainfall, temperature, relative humidity and solar radiation) of the trial site are shown in Fig. 2.1.

A rust susceptible cultivar EL-142 was used for the study. To minimize the transfer of inoculum from plot to plot, the plots were made square (Paysour and Fry, 1983), and each plot measured 3 m x 3 m. Seeds were sown at 5 cm distance within a row with 30 cm distance between rows. A path of two meters was kept between plots and replications, and each plot was surrounded by five guard rows (20 cm between rows) of durum wheat to minimize interplot interference (Aust and Kranz, 1988).

Treatments were evaluated in a randomized complete block design with six replications. In order to create different rust epidemics, the treatments consisted of four frequencies of application of the systemic triazole fungicide Folicur® 250 EW (tebuconazole) (Marcellos, Moore and Nikandrow, 1995) at a rate of 500 ml/ha plus a wetting-spreading agent (Foliwett® 900, 10 ml/100 l H_2O). The fungicide was applied at intervals of five, 10, 15 and 20 days. An unsprayed check was included as a control treatment. The fungicide applications were started one week after inoculation. When spraying the fungicide, a strip of plastic was held against the wind direction to limit fungicide drift between plots. The experimental plots were hand weeded twice (20 and 60

days after sowing) and fertilised with nitrogen at the rate of 20 kg N/ha at the seedling stage. The insecticide dimethoate (Roger® 400 EC) at the rate of 1 litre per ha was applied for the control of pea aphid (*Acyrthosiphon pisum* Harris).

Four weeks after sowing, each plot was inoculated by spraying with *ca*. 1 litre of a suspension containing 250 mg urediniospores of lentil rust collected from Akaki in 2000. The concentration of the spore suspension was determined using a haemocytometer to be about 2 x 10^5 urediniospores ml^{-1}, i.e. 6.6 times more than that (3 x 10^4 ml^{-1}) suggested by Kramm and Tay (1984). The concentration was increased to ensure success of infection. The spore suspension was sprayed using a knapsack sprayer. Tween® 20 (polyoxyethylene sorbitan monolaurate) was added as wetting agent at a rate of one drop per 1000 ml of the suspension (Chongo and Bernier, 1999). Wooden frames were placed over inoculated plots and covered with clear polyethylene sheets overnight and uncovered the next morning (Hanounik, 1986; Chongo and Bernier, 1999). Moist soil and plastic sheeting created a high humidity environment (> 80% RH) for infection.

Twelve plants of the four central rows, avoiding three border rows on either side of a plot, were randomly selected and tagged permanently for disease evaluations. These tagged plants were assessed on each observation day for rust severity, incidence, pustule size and pustule density. Disease assessment started a week after inoculation and continued until maturity at weekly intervals. A total of eight assessments were made during the entire crop life. On each assessment date, rust severity was scored in the upper, middle and lower leaf canopy layers separately on each of the 12 sample plants. Rust severities (RS) were scored on a 1-9 scale (Khare, Bayaa and Beniwal, 1993). These

scores were then converted to percentage severities using the following formula by Chongo, Bernier and Buchwaldt (1999):

$$\%RS = \frac{\sum[no.of\ plants/scale \times scalevalue]}{highest\ scale \times total\ no.of\ plants} \times 100$$

Disease incidence was measured as the percentage of leaves with detectable rust disease per plant. Similarly, pustule size on a 4-point scale (Conner and Bernier, 1982) and pustule density as number of pustules/cm^2 leaf area were measured per plant. All leaves of a whole sample plant with all pustules on them were considered for determining pustule density.

Crop growth stages were assessed on the dates of disease evaluation following the Erskine, Muehlbauer and Short (1990) growth stage key (Table 2.1). The leaf area of each plant selected for disease assessment was estimated weekly (on each disease assessment date) by means of calibrated grids printed on transparent sheet (Zadoks and Schein, 1979). The leaf area index (LAI), based on the weekly measurements, was calculated as the ratio of leaf area per cm^2 ground area to cm^2 ground area. Days to maturity (number of days from planting to physiological maturity, i.e., when pods turn brown and leaves and stems become dry) were also recorded.

At full maturity, seed yield in g per m^2, seed mass in mg per seed, number of pods per plant and number of seeds pod^{-1} of the four central rows were assessed. Pods per plant and seeds per pod were counted at harvest. Before threshing, biological yield was determined by weighing total biomass of plants harvested from each plot. Seed yield and seed mass were determined after threshed seeds were sun dried between 08:00 and17:00

daily for 7 days. Harvest index was calculated as seed mass or yield per m^2 per plot divided by mass or yield of whole plants per m^2 per plot.

Area under the disease progress curve (AUDPC) was calculated using the formula by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n} (X_i + X_{i+1}) / 2(t_{i+1} - t_i)$$

in which X_i = rust severity at the *i*th observation, t_i = time (days) at the *i*th observation, and n = total number of observations.

By analogy to area under the disease progress curve, areas under the disease incidence and the crop growth progress curves were calculated using the formula given above. The variable rust severity was replaced by the variable of interest.

The raw severity data (72 data sets per assessment date and canopy layer) were averaged per plot for each assessment date and canopy layer and these were used to establish a relationship between disease and seed yield. In order to develop yield loss models, the severity data were analysed using the most commonly used groups of disease-yield models (Zadoks and Schein, 1979; Madden, Pennypacker and Kingsolver, 1981; Sache and Zadoks, 1994; Madden and Nutter, 1995). These included (1) critical point models, which relate yield to severity at a single assessment date; (2) multiple point models, which relate yield to disease severities at more than one assessment date; and (3) AUDPC models, which relate yield to AUDPC. In the latter, the whole range of AUDPC values truncated at different times after sowing and the complete disease curve were considered as model inputs.

The dependent variables were seed mass, seed yield per plant and seed yield per unit area. The independent variables for the critical point and multiple point models were rust severity on the upper, middle and low canopy layers and average of the three layers for each of the eight assessment dates (*t*) (time, expressed as days after sowing). The independent variables for the AUDPC models were final AUDPC values and cumulative ones between the first assessment date and *t* on the upper, middle and lower canopy layers as well as AUDPC values averaged over the three layers. Regression models were used to study the relationship between the dependent and independent variables. Data transformations were applied to independent variables to investigate non-linear relationships, but that did not improve model fit.

Variance ratios for crop growth parameters and for each of the disease parameters at the different crop growth stages and canopy layers were computed (cross-sectional analyses). Simple correlations were calculated among yield components, and between rust parameters and yield components.

Analysis of variance (ANOVA) was used to investigate the effects of treatments on the temporal spread of lentil rust epidemics and crop growth. Treatment means of apparent infection rate (r_L), i.e. regression coefficient *b* and AUDPC, time for the rust to reach 10% severity (T_{10}) and area under the disease incidence progress curve were used to characterize and compare the temporal epidemic progression (spread of lentil rust epidemics). The least significant difference test was used to determine treatment differences at $P \leq 0.05$ and/or $P \leq 0.01$. Data analyses were performed using the MSTAT-C statistical software (Bricker, 1991). Excel graphics software (Microsoft Excel 2000) was used to produce the graphs.

RESULTS

Treatment differences for LAI were not significant at any of the crop growth stage (Table 2.2). There were highly significant ($P \leq 0.01$) treatment differences in rust incidence at all growth stages of the crop except for the non-significant treatment difference at V11 (Table 2.2). The maximum level of rust incidence (71%) recorded was in unsprayed control treatment at R6.

In plots with maximum rust infections, rust severity reached its highest level of 43% and 79% in the upper canopy layer at R5 and middle canopy layer at R4, respectively, in the untreated check treatment. Differences between treatments were significant ($P \leq 0.01$) at almost all crop growth stages and all canopy layers (Table 2.2).

Rust started and symptoms became conspicuous on the lower canopy layer for most parts of the epidemic duration. The profiles (distribution) of rust in the canopies of the unsprayed control treatment are shown in Fig. 2.2. The values of gradients for upward (vertical) distribution of rust severity are presented in Table 2.3. Simple linear regression model described the data satisfactorily at growth stages R1 and R2. Estimates of *b* were -0.5 and -0.4 at the vegetative stage V13 and first flowering stage R1, respectively. These values are lower than those at R2-R5. The differences between treatments were significant ($P \leq 0.05$) for AUDPC at all crop growth stages in all the canopy layers (Table 2.2). Differences between treatments for AUDPC values averaged over the three canopy layers were also significant ($P \leq 0.05$) at all growth stages.

Differences between treatments for pustule density were significant from R1 to R6 (Table 2.2). Average pustule density ranged from 0.02 pustules per cm^2 to four pustules per cm^2 leaf area, the maximum being for the unsprayed control treatment (Fig.

2.3A). Treatment differences for pustule size were significant at all crop growth stages (Table 2.2). The largest pustule size observed was in the untreated control treatment (maximum rust infection plot) (Fig. 2.3B).

Differences between rust epidemics for seed yield were significant ($P \leq 0.05$). Treatments 3 (spray every 10 days) and 4 (spray every 5 days) significantly ($P \leq 0.05$) increased seed yield over the control, treatment 0 (no spray). The seed yield from treatment 3 (spraying every 10 days) was significantly ($P \leq 0.05$) higher than the yield from treatment 1 (spraying every 20 days, high rust (Fig. 2.4A). No significant yield differences were found between treatments 4, 3 and 2 (spray every 15 days). Treatment 4 (spraying tebuconazole every 5 days) caused a slight decrease in seed yield over treatment 3 (spraying tebuconazole every 10 days). Seed yield ranged from 73 g m^{-2} for no spray to 125 g m^{-2} for treatment 3 (spray every 10 days). The range of variation was 52 g m^{-2}.

Rust epidemics significantly ($P \leq 0.05$) affected seed mass and pods per plant (Figs. 2.4B, 24D). Generally, seed mass increased with decreased rust infection. Seed mass varied from 19 mg per seed for the untreated control treatment (maximum rust infection plot) to 25 mg $seed^{-1}$ for treatment 4 (relatively rust-free plot). In contrast to this, rust infections did not affect seeds per pod (Fig. 2.4C). Although treatment 1 (spray every 20 days) resulted in a small enhancement in this yield component, there was no regular pattern of variation among treatments.

Significant ($P \leq 0.05$) treatment differences were also found for pods per plant (Fig. 2.4D). The treatment with fungicide application at five days interval had slightly less pods per plant when compared with other sprayed treatments (Fig. 2.5). Rust

significantly ($P \leq 0.05$) decreased the number of days to maturity and harvest index (Table 2.4). Days to maturity were in the range of 101-116 days. The coefficient of linear regression of seed yield on days to maturity was found to be 3.2; SY = -252.5 + 3.2DM, where SY, seed yield; and DM, days to maturity.

Of the 15 linear correlation coefficients for the relationship between yield components, only seven of them were significant. Of these, seed yield showed a positive and significant association with number of pods/plant, seed mass and days to maturity, and the correlation coefficients (r values) were higher for the relationship between seed yield and pods/plant and days to maturity (Table 2.5). This suggests the significance of these components in the determination of seed yield in lentil cv. EL-142. When seed yield per plant (SYPP) was regressed on the product of pods per plant (PP), seed mass (SM) and seeds per pod (SP), the following equation was obtained.

$SYPP = 0.43 + 0.86_{PPSPSM}$, $R^2 = 0.98$; SE = ± 0.055

The linear effect of the product of pods per plant, seeds per pod, and seed mass contributed significantly to the variation in seed yield, because the estimated simple linear regression, $SY = 0.43 + 0.86_{PPSPSM}$ was highly significant ($P \leq 0.01$). Moreover, 98% of the total variation in lentil seed yield was explained by the linear function of the product of pods per plant, seeds per pod, and seed mass. The regression equation estimated seed yield with a standard error of only 0.055 (± 5.5% of the estimate). In testing the significance of the regression coefficient (β or the slope the regression line), the computed t-value (13.93) is greater than the required t-value (5.4) at the 1% level of

significance. Therefore, there is a linear response of lentil seed yield to changes in the three components.

From results of the regression analysis stated above, lentil yield may be described by the general equation,

SY = PP*SP*SM

Where SY, seed yield; PP, pods per plant; SP, seeds per pod; and SM, seed mass.

The correlation coefficients for rust incidence and yield components and yield contributing characters are given in Table 2.6. The correlation between rust incidence with seed mass, harvest index and time to maturity were significant ($P \leq 0.05$) at most of the crop growth stages. The correlation coefficients between rust severity and seed yield showed variation among growth stage and canopy layers (Table 2.7). Significant r-values were obtained in the upper and middle canopy layers at R1 and R3 growth stages. The values of r ranged from -0.83 to -0.90, the highest (-0.90) being for the upper canopy layer at R1 growth stage.

The relationship between rust severity and seed mass also showed variation among leaf canopy layers and growth stages. There were significant ($P \leq 0.05$) and high r-values in almost all canopy layers and growth stages. As opposed to seed yield and seed mass, the correlations between rust severity and seeds per pod were non-significant in any of the canopy layers and growth stages.

The number of pods was significantly reduced by rust and its correlation with rust severity was significant in most of the canopy layers and at almost all of the crop growth stages only when treatment 4 is taken out of the correlation analysis (data not shown).

This was so because treatment 4 (spraying tebuconazole every five days) slightly decreased the number of pods per plant in spite of its low disease severity (Fig. 2.4D). Otherwise, the correlation between rust severity and this component was non-significant in all canopy layers and crop growth stages (Table 2.7)

The relationship between rust severity and harvest index was similar to that for rust severity and seed yield, i.e. growth stage-canopy-layer specific relationship. The values of r were significant only in the upper and middle canopy layers at R3 and R4 growth stages (Table 2.7). Correlations between rust severity and days to maturity were negative and the correlation coefficients were significant only at R1-R4 growth stages in all the canopy layers (Table 2.7).

The progress of LAI and the area under LAI progress curve for each epidemic are shown in Fig. 2.6A and Fig. 2.6B, respectively. There were no significant effects of rust infection on these elements of crop growth (Table 2.8). In all the treatments LAI increased up to 65 days after sowing and reached a plateau or decreased thereafter. Area under the LAI progress curve varied from 45 to 51 LAI-days.

The rust incidence progress curves and thus the area under rust incidence progress curve varied markedly for each epidemic (Fig. 2.7A and Table 2.8). In the unsprayed control plot, the epidemic reached a 60% incidence. The incidence remained very low in treatment 4 (sprayed every five days). In general, incidence increased with time. All the epidemics started with the same level of rust incidence, but the pattern of incidence progression was different for the different treatments/epidemics. The rust severity progress curves for each treatment/epidemic averaged over the three canopy layers are shown in Fig. 2.7B. The starting point of the curves was the same, but as time went by

their pattern of temporal progression and shape differed with treatments/epidemics. The progress of rust in the three canopies of the different treatments/epidemics is shown in Figs. 2.8A-E. Rust severities were initially at a very low level and increased with time in each canopy and treatment (Figs. 2.8A-E). The graphs show the continuous infection of the leaves in the upper canopy layer.

The temporal progression of the rust varied with treatments (Fig. 2.7B). Comparison of this variation was made possible through the analysis of apparent infection rate, which is equivalent to the slope of the logit severity line against time (Fig. 2.9). Linear regression lines were fitted to the scatter points in each treatment, and the percentage of total variability explained by the regression was high (≥ 95%) for all the treatments except for treatment 4. The regression coefficients had low standard errors and short 95% confidence intervals (Table 2.9). All of the regressions and their estimates (*b* values) were significant at the 1% level. The epidemics were, therefore, best decribed by the regression equation of the general formula $log_e[x/(1-x)] = a + bT$, where x = the proportion of leaf area infected by rust and T = time (Zadoks and Schein 1979).

Averaged over all canopies, the rate of temporal progression of the disease or apparent infection rate differed significantly ($P \leq 0.05$) among the different epidemics (Table 2.9). Apparent infection rate (r_L) for the unsprayed control treatment (maximum rust infection plot) was 2.36 times greater than r_L for a treatment sprayed every five days (a relatively rust-free treatment or plot). Irrespective of frequency of application, tebuconazole spray decreased r_L significantly ($P \leq 0.05$).

Time required for severity to reach 10% (T_{10}) increased as frequency of application increased. However, this was not always true. Values of T_{10} for the 15-day

interval were significantly greater than T_{10} for the unsprayed control, whereas the 10-day interval did not significantly increase T_{10} when compared to the unsprayed control (Table 2.9). This indicates that T_{10} is less reliable than r_L in analysing and comparing disease progress or dynamics of lentil rust epidemics under Akaki conditions. The value of T_{10} for the 5-day interval was significantly higher than T_{10} values for other treatments. The shortest T_{10} was that of unsprayed control. Forty-six more days were required for rust severity to reach 10% in the plots sprayed every five days than in unsprayed control plots.

The AUDPC values for the control treatment were 950, 2309, and 2304%-disease days in the upper, middle and lower canopy layers, respectively. These AUDPC values were significantly ($P \leq 0.05$) higher than the respective AUDPC values of the fungicide-treated plots (Table 2.8). Averaged over treatments, the ratio of the AUDPC for the lower canopy to the middle and upper canopies, respectively, were 1.13 and 2.55. The ratio of the AUDPC for the middle canopy to the AUDPC for the upper canopy was 2.24.

Seed yield losses ranged from 15.8 to 39.2 % (Table 2.10). Regression analysis showed that seed yield of EL-142 was reduced by as much as 8.39% for each unit increase in rust severity in the upper canopy layer at the first bloom (R1) growth stage (Table 2.11). The decrease in seed yield, either per plant or unit area, related to disease severity/AUDPC on the upper or middle canopy layer, occurred mainly between days 51 and 65 days after sowing (R1-R3), i.e. early flowering (R1) to early pod formation (R3) (Table 2.12). Seed yield loss linearly increased with increasing disease severity on the UC during R1 (Fig. 2.10A). The seed yield loss was described equally well by using the complete AUDPC values at R6 crop growth stage. The yield loss models/equations indicated in Table 2.11 accounted for 80% or more ($R^2 \geq 0.80$) of the explained

variability in lentil seed yield loss. The models that fitted seed mass x canopy layer x time data are shown in Fig. 2.11. There was a sharp decline in seed mass with a slight increase in disease severity in the upper canopy layer at 51 days after sowing. In middle and lower canopy layers at 51 days after sowing, the decline or decrease in seed mass was not as sharp as in upper canopy layer.

Significant relationships between yield components and rust disease severity and AUDPC on the upper, middle and lower canopy layers were found (Table 2.12). Not all univariate and multivariate models tested for each component x canopy layer x time combination were significant. Therefore, the models giving significant relationships were selected (Table 2.12).

A significant negative correlation between yield/yield components (except pods per plant and seeds per pod) and independent variables was found. However, the strongest decrease in yield /yield component (except the seed mass per plant) was related to disease severity on the upper canopy layer on day 51 (R1 or first blooming) (Table 2.12 and Fig. 2.10A).

The relationships between days to maturity and rust severity on the different canopy layers at different growth stages are shown in Table 2.13. The coefficients of regression of days to maturity on rust severity ranged between -0.182 for rust severity in the middle canopy layer at R4 and -3.02 for rust severity in the upper canopy layer at the R1 growth stage. This indicates that the time to maturity decreased by 0.182 to 3.02 days for every 1% rust severity.

DISCUSSION

Application of tebuconazole at varying intervals successfully created different field rust epidemics. This was reflected by the differential influence of the spray treatments on rust parameters. Although spray treatments did not have a significant effect on leaf area index at any of the crop growth stages, they significantly affected rust parameters. Clearly, this shows that rust had little effect on lentil plant growth. Such little effect of *U. viciae-fabae* on plant growth has been reported in faba bean (*Vicia faba* L.) as well (Williams, 1978; Marcellos *et al.*, 1995).

Results of this study can identify the most effective time to spray fungicides against rust in lentil, i.e., between flowering and seed filling. This is consistent with Marcellos *et al.* (1995) who reported that mancozeb applied at early and late flowering growth stages controlled *U. viciae-fabae* and increased grain yield in faba bean. However, despite use of fungicides as a rust control option in lentil production under the present Ethiopian conditions not being realistic, the prospects of including it in a rust management program are positive.

Leaves on the lower canopy layer had a rather early and severe rust attack. Such a disease distribution pattern is a characteristic feature of endogenous epidemics or development of a disease from an endogenous source, i.e. when the source of infection is within the infected plant itself (Daamen, 1989). Factors such as high relative humidity for the lower canopy increases the chances of further leaf infection compared to the leaves of upper canopy layer as is the case with cereal crops (James and Shih, 1972).

Lower leaves, under circumstances mentioned above, may also serve as a source of infection for the leaves in the middle and upper nodes. Early inspection of lower leaves

for rust infection may provide information which can be used to control infection higher up in the canopy. Habtu and Zadoks (1995) reported on the epidemiological significance of lower leaves. Dividing the lentil plant into different canopy layers and assessing rust disease for each layer separately may be helpful to understand the spatial (vertical) spread of the disease.

In lentil, the most important components of seed yield and yield-contributing characters are pods/plant, seed mass, pods/peduncle, time to maturity, and plant height (Balyan and Singh, 1986; Kumar and Bajpai, 1993). Data from this study, too, revealed that seed mass, pods per plant and days to maturity had positive and significant correlations with seed yield. Although correlations are not indicative of cause and effect relationships, they can disclose the type of associations between variables. Thus any stress having a negative association with one of these yield components and yield contributing characters may also have a negative relationship with seed yield. In this study, it was shown that lentil rust had significantly reduced seed mass, numbers of pods per plant, harvest index and shortened the time to maturity. In the present study, pods per plant were positively and significantly correlated with harvest index. Such a pattern of character association has been reported by Singh (1977) and Balyan and Singh (1986).

Lentil rust at early flowering growth stage might have affected the number of fertile flowers and finally the number of pods. The rust also reduced seed size (mass). The effect of rust on reduced final grain size could be due to shortened longevity of the pod-filling period by accelerated maturity or early termination of growth. The phenomenon of accelerated crop development due to high temperature, and its effect on grain size in lentil was reported by Dutta *et al.* (1993).

A negative, significant correlation between rust intensity (severity and incidence) and time to maturity suggests the rust disease as being a factor responsible for early termination of crop growth/development. Hence, *U. viciae-fabae* could be considered as a growth terminator, i.e., a biotic factor causing forced maturity in lentil. Under Akaki conditions, cultivar EL-142 matures in about 110 days after sowing (Bejiga, Million and Yadeta, 1995). In this study, infected plants of this cultivar matured in about 100 days.

Evidence from this study indicates that if rust is controlled then the number of days to maturity is increased. Lengthening the maturity period will, in turn, result in increasing seed yield at the rate of 3.2 g m^{-2} day^{-1} or 31.8 kg ha^{-1} day^{-1}, under environmental conditions that prevailed at the study site. Khan *et al.* (1998) and Metzer, Czaplelwski and Rasmusson (1986) reported the advantage of an increased maturity period in lentil and barley, respectively.

Seed mass was the yield component most sensitive to rust attack, as was shown in the analysis of variance. Seeds per pod was not affected. These results are consistent with those of Marcellos *et al.* (1995), Rashid and Bernier (1991) and Yeoman, Lapwood and McEwen (1987) who reported that loss in faba bean yield due to rust (*U. viciae-fabae*) was due mainly to reduced seed mass. The average seed mass from rust free lentil plants was about 25 mg $seed^{-1}$ as opposed to 19 mg $seed^{-1}$ from rusted plants. The seed mass reported for the cultivar EL-142 is 22 mg $seed^{-1}$ (Bejiga *et al.*, 1995).

The association of rust intensity with pods per plant was significant under different conditions (leaf canopy layer and crop growth stage). These results corroborate the findings of Habtu and Zadoks (1995) in an experiment with rust [*Uromyces appendiculatus* Pers. (Unger)] in haricot bean (*Phaseolus vulgaris* L.). Of all the yield

components and yield-contributing characters, seed mass and days to maturity were most sensitive to rust attack in lentil.

Progress of the rust epidemic followed the general pattern of host growth curve or progress of leaf area index. There was a sharp rise, linearity and leveling-off in the rust progress curve at the start, intermediate and terminal parts of the epidemics, respectively. This similar pattern of lentil crop growth and rust progress curves indicates that the change in lentil growth had an effect on rust disease development. Van der Plank (1963) explained how changing host quantity affects the development of the epidemic.

In the bean-rust (*P. vulgaris-U. appendiculatus*) pathosystem, significant reduction of leaf area due to rust has been reported by Habtu *et al.* (1997). Therefore, the effect of *U.* v*iciae-fabae* on leaf area index of lentil needs to be studied in more detail before conclusions regarding its usefulness in analysing rust epidemics and comparing the effects of different rust control practices are made.

The rust incidence progress curve under high disease pressure may be quite different from that of a curve in a low disease situation. The incidence progress curve in the plot with maximum rust infection was of the type what is referred to as S-shaped by Kranz (1978) or in that particular family of curves. The processing of several consecutive severity assessments at equal time intervals (longitudinal study) yielded three different elements or statistics associated with disease progress curves, and helped to make comparison in lentil rust epidemics (Zadoks, 1972; 1978). The three statistics included the apparent infection rate (r_L), AUDPC and time required for rust severity to reach 10% (T_{10}). The (r_L) value was calculated as the slope (b) of a straight line fitted to the points of logit severity plotted against time for each treatment. Linearity was achieved in ln [x/(1-

x)] plotted against time (Fig. 2.9). The apparent infection rate estimated the true infection rate of lentil rust accurately, because the coefficients of determination (R^2) associated with the calculation of r_L were significant and high (> 0.80). Apparent infection rate or the rate at which lentil rust increases under Akaki conditions may, thus, be best described by the logistic equation $dx/dt = ln\{[x/(1-x)]-a\}/t$, where $dx/dt = b$ or the linear regression coefficient, representing the apparent infection rate (r_L).

The importance of mid-way time, the time at which 50% of the epidemic process is completed (T_{50}) in longitudinal studies, has been pointed out by Zadoks (1972). The T_{10} used in this study, however, offers an advantage over T_{50} because the T_{50} values of some disease control treatments may fall outside the growth cycle of the host crop. A rust epidemic cannot reach mid-way time in treatment 4 as this was evidenced by the T_{50} that fell far beyond crop maturity. Therefore, T_{10} seems a more realistic parameter to characterize and compare the time course of epidemic development under various chemical spray treatments. Nonetheless, it was no better than r_L in making distinctions among treatments. This was so because both the disease progress curve elements were calculated from the same regression equation (logit $Y = a + bt$) for each treatment and the values of the position parameter (the *Y*-intercept or *a*) of the transformed disease progress curves were nearly the same for all treatments (Fig. 2.9).

AUDPC was calculated from the untransformed severity data. The AUDPC reveals the true variation in disease development and ANOVA for this statistic is, thus, expected to detect differences more than ANOVA for r_L or T_{10}, because AUDPC is free from transformation effect and its calculation is independent of the regression equation (Shaner and Finney, 1977). In this study, however, AUDPC did not discriminate

treatments more than did r_L or T_{10}. This could possibly be due to the little variation observed among the spray frequencies. The three statistics were helpful to analyse the different epidemics and to compare treatment effects on disease development. Therefore, all three may be considered as criteria to compare lentil rust epidemics and the effects of rust control practices. The objective of comparison and ease of calculation has to be considered in choosing one of the three.

The forced maturity of rust infected plants prevented the crop from making full use of the resources available during the growing season eventually leading to reduced seed size and yield. In other words, seeds in diseased plants stopped growing earlier than the normal maturity date. As a result, the final seed mass was significantly decreased when compared to the seed mass harvested from healthy plants. The smaller mass recorded in diseased plants could be due to reduced availability of assimilates (Shtienberg, 1990).

Physiological studies have shown that the critical stage for pod setting occurs about two weeks after the initiation of the flowering period (Chandra and Asthana, 1988). According to them, about 80-90% of lentil pods are formed during this stage, and seed mass and number of seeds per pod are the highest during this period. This suggests that any adverse condition during this stage could greatly reduce productivity in lentil. In the present experiment, rust disease significantly decreased seed mass between days 51 and 65, which is the critical stage for pod setting, i.e. the stage at which lentil plants accumulate yield to the maximum and are very much sensitive to rust. In terms of physiological development, this stage is between R1 and R3. In other crops such as

wheat, for instance, most carbon for grain filling is assimilated after anthesis (Agrawal and Sinha, 1984).

In this study, seed mass was found to be the primary component of seed yield loss. A foliar disease such as brown spot (*Septoria glycines*) of soybean has been shown to cause yield loss in *Glycine max* in a similar manner (Hartman *et al.*, 1987). Shtienberg (1990) reported that leaf rust substantially decreases final seed mass in wheat.

The rust disease developed rapidly and reached a high severity four to five weeks after the initiation of the epidemic, i.e, about three disease cycles (Fig. 2.7B), and occurred mainly between the time of flowering and pod formation stage, indicating that the epidemic of lentil rust is of short duration. A similar observation has also been made for *U. viciae-fabae* on faba bean (Sache and Zadoks, 1994). Critical point models are usually superior to other models in describing disease-yield relationships under short epidemic conditions (Campbell and Madden, 1990). In this study, critical-point models (Tables 2.12 and 2.13) best predicted the effects of rust on yield/yield components of lentil. Sache and Zadoks (1994) made similar conclusions for the faba bean-rust pathosystem. Data of the present study showed that the critical stage during which seed yield and seed mass were significantly affected by the rust coincided with the linear phase of the disease progress (Fig. 2.7B). A reduction in seed mass was reported as the main effect of rust by Lapwood *et al.* (1984), Rashid and Bernier (1991), Sache and Zadoks (1994) in faba bean and Habtu *et al.* (1997) in haricot bean.

Regression of lentil seed yield on rust severity showed that seed yield was reduced by 8.39% for every 1% increase in disease severity on the upper canopy layer during the critical stage, R1 (Fig. 2.9A). In terms of absolute value, this percentage loss

was equivalent to 10 kg seed yield reduction per ha for every 1% increase in disease severity. This figure is 1.5 kg lower than that reported by Singh and Jhooty (1986) and could be attributed to several factors such as differences in location, genotype and crop growth stage. Singh and Jhooty (1986), for instance, did not specify the crop growth stage at which the rust assessment was made. The work of Brooks (1972) showed that if mildew attacks spring barley early in the season, it is not possible to correlate yield loss with mildew severity at a late growth stage, because the losses for the two growth stages with the same percentage severity are different. Rust severity $\geq$ 4.7% during the critical stage would significantly decrease final seed yield of lentil (see Appendix 2.1), and severity values at this stage seem more important than the severities at any other stage for the purpose of yield loss assessment. The 4.7%-severity level could be taken as action threshold level. Kim and McKenzie (1987) defined action threshold level as a point beyond which control strategy is initiated. This level is also referred to as the critical level (damage threshold) (Nutter *et al.*, 1993). Therefore, rust assessment at this critical stage would be advisable in making disease control decisions. However, this may vary between locations.

Of the three types of models tested, yield loss in lentil due to rust may be described and predicted using critical point and AUDPC models as their R^2 values fell within the acceptable range. For crop loss studies, R^2 values ranging from 0.8 to 0.9 are generally considered excellent (Madden, 1983). Moreover, the respective coefficients of regression (t-statistic of *b*) were significant ($P \leq 0.05$). This indicates that there is a linear response of lentil yield loss to changes in the level of rust infection within the range of 0 to 4.7% rust severity in the upper canopy layer at the R1 growth stage. The maximum

possible yield loss recorded in this study was 41.7%. The possible effect of disease severity in the lower layers on seed yield is not considered important, because, physiologically, the lower leaves are believed to have little effect on yield (Griffiths, 1984).

For the critical point loss model at R1 ($Y = 2.22 + 8.39x$), visual inspection of the scatter diagram or the scatter plot of residuals with the predicted yield loss values revealed a random and homogenous variance for yield and rust severity (the residual values were almost constant with the exception of one outlier) (Fig. 2.10B). This shows that the error term (μ) in the generalized linear loss model/equation: $Y = \alpha + \beta X + \mu$ has a normal distribution. The regression equation, therefore, fulfils the assumptions required by the procedure of regression analysis. Thus, this loss model is significant, reliable and useful.

The yield loss prediction models that were developed from the disease database generated in the present study might be used for lentil yield loss assessment and rust disease control decision purposes in areas with environments similar to Akaki in Ethiopia. However, the models need to be validated before they are put in practice. The validation may be done at simple-cross or double-cross validation levels as described by Madden (1983).

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Table 2.1 Growth stage descriptions for lentil (Erskine *et al.* 1990)

Stage	Abbreviated stage title	Description of growth stage
V1	One node	A plant with one node or a plant at vegetative stage V1
V2	Two nodes	A plant with two nodes, in vegetative stage V2
Vn	N number of nodes	A plant with n number of nodes in vegetative stage Vn
R1	First bloom	One open flower at any node
R2	Full bloom	Flower open or has opened on nodes 10-13 of the basal primary branch
R3	Early pod	Pod on nodes 10-13 of the basal primary branch visible
R4	Flat pod	Pod on nodes 10-13 of the basal primary branch has reached its full length and is largely flat. Seeds fill less than half of the pod area but can be felt as a bump between the fingers
R5	Full seed	Seed in any single pod on nodes 10-13 of the basal primary branch are swollen and completely fill the pod cavity
R6	Full pod cavities	All the normal pods on nodes 10-13 of the basal primary branch are swollen and completely fill the pod cavity
R7	Physiological maturity	The leaves start yellowing and 50% of the pods have turned yellow
R8	Full maturity	90% of pods on the plant are golden-brown

Table 2.2 Cross-sectional analysis of effects of treatments (fungicide spray frequencies) on leaf area index (LAI) of lentil, rust incidence, severity, pustule density (PD) and size (PS) measured at different lentil growth stages and leaf canopy layers at Akaki, Ethiopia

Variables	Source of variation	Variance ratio values at growth stage (GS)							
		V11	V13	R1	R2	R3	R4	R5	R6
LAI	Replication R)	ns[a]	3.6*	ns	3.9*	3.2*	3.7*	3.4*	3.4**
	Treatment (T)	ns	Ns	ns	ns	ns	ns	ns	ns
Incidence	R	2.8*	6.4**	ns	ns	4.9*	ns	3.6*	ns
	T	ns	14.0**	15.0**	97.2**	37.3**	45.3**	43.3**	31.0**
Severity-UC[b]	R	ns	3.6*	ns	ns	ns	ns	ns	ns
	T	ns	4.9**	7.0**	17.1**	42.0**	39.1**	36.2**	32.0**
-MC	R	ns	Ns	ns	ns	ns	ns	ns	4.6**
	T	ns	5.5**	10.5**	32.5**	ns	56.8**	83.3**	56.0**
-LC	R	ns	Ns	ns	ns	ns	3.2*	ns	3.4*
	T	ns	6.0**	13.5**	32.2**	39.2**	90.6**	55.9**	63.7**
- Average	R	ns	Ns	ns	ns	ns	ns	2.8*	4.2**
	T	ns	7.3**	16.9**	40.6**	85.6**	117.7**	107.2**	84.8**
AUDPC-UC	R	—	—	ns	ns	ns	ns	ns	ns
	T	—	—	6.7**	10.0**	21.7**	49.9**	66.0**	74.4**
-MC	R	—	—	ns	ns	ns	ns	ns	ns
	T	—	—	5.7**	11.0**	81.8**	74.4**	75.4**	77.9**
-LC	R	—	Ns	ns	ns	ns	ns	ns	ns
	T	—	4.3*	10.4**	29.8**	38.9**	51.7**	61.4**	63.5**
- Average	R	—	Ns	ns	ns	ns	ns	ns	ns
	T	—	4.0*	7.9**	14.6**	76.2**	101.9**	112.4**	110.4**
PD	R	3.7*	Ns	ns	ns	ns	ns	ns	ns
	T	ns	Ns	23.6**	66.2**	62.4**	65.5**	52.6**	44.4**
PS	R	ns	4.6**	ns	ns	2.73*	ns	2.8*	3.0*
	T	ns	10.1**	14.5**	16.6**	60.2**	28.0**	50.4**	51.2**

Level of significance: *, $P \leq 0.05$; **, $P \leq 0.01$ (ANOVA).

[a] ns, not significant; —, not determined.

[b] UC, upper canopy layer; MC, middle canopy layer; LC, lower canopy layer.

Table 2.3 Estimates of gradients of distribution of rust severity in leaf canopy layers (slopes of the regression of rust severity on distance from lower canopy) at different lentil growth stages together with the corresponding coefficient of determination R^2 and significance of the regression equation

Statistical parameter	Growth stage					
	V13	R1	R2	R3	R4	R5
Intercept (a)	0.83	0.99	1.00	1.2	1.15	1.10
Gradient or slope (b)	-0.50	-0.40	-0.36	-0.30	-0.23	-0.26
R^2	0.75	0.99	0.99	0.42	0.44	0.67
Significance[a]	ns	**	**	ns	ns	ns

[a]ns, not significant at 5% level of probability; **, significant at 1% level of probability.

Table 2.4 Effect of rust on time to maturity and harvest index of lentil

Spray interval (days)	AUDPC (%-disease days)	Time to maturity (days)	Harvest index
5	203	116.0	0.52
10	471	116.0	0.54
15	875	114.0	0.56
20	980	109.0	0.54
No spray (control)	1854	101.0	0.38
LSD.05	158	13.0	0.05
S.E. (±)	76	1.1	0.05
C.V. (%)	14	1.0	7.80

Table 2.5 Correlation matrix of pods per plant (PP), seeds per pod (SP), seed mass (SM), days to maturity (DM), harvest index (HI) and seed yield (SY) of lentil cultivar EL-142

	PP*	SP	SM	DM	HI	SY
PP	1.00					
SP	ns	1.00				
SM	ns	ns	1.00			
DM	ns	ns	0.96	1.00		
HI	0.92	ns	0.87	0.86	1.00	
SY	0.93	ns	0.84	0.94	ns	1.00

* ns = not significant at the 1% level of probability.

Table 2.6 Linear correlation coefficients between rust incidence and yield components in lentil. GS, growth stage; SY, seed yield; SM, seed mass; SP, seed per pod; HI, harvest index; and DM, days to maturity

GS	SY	PP	SM	SP	HI	DM
V11	ns	ns	Ns	ns	ns	ns
V13	ns	ns	-0.91	ns	ns	ns
R1	Ns	ns	-0.97	ns	ns	-0.91
R2	Ns	ns	-0.97	ns	-0.88	-0.91
R3	Ns	ns	-0.95	ns	ns	-0.90
R4	Ns	ns	-0.97	ns	-0.88	-0.91
R5	Ns	ns	-0.98	ns	-0.92	-0.94
R6	Ns	ns	-0.99	ns	-0.89	-0.95

* ns = not significant at the 1% level of probability.

Table 2.7 Linear correlation coefficients between rust severity and yield components in lentil

Leaf layer	Growth stage (GS)	Seed yield (SY)*	Seed mass (SM)	Seed/pod (SP)	Pod/plant (PP)	Harvest index (HI)	Days to maturity (DM)
Upper canopy	R1	-0.90	-0.88	ns	ns	ns	-0.92
	R3	-0.85	-0.97	ns	ns	-0.87	-0.94
	R4	ns	-0.93	ns	ns	ns	-0.88
	R6	ns	ns	ns	ns	ns	ns
Middle canopy	R1	-0.83	-0.98	ns	ns	ns	-0.94
	R2	ns	-0.97	ns	ns	ns	-0.92
	R3	-0.88	0.95	ns	ns	-0.90	-0.92
	R4	ns	-0.94	ns	ns	-0.85	-0.89
	R5	ns	-0.90	ns	ns	ns	ns
	R6	ns	ns	ns	ns	ns	ns
Lower canopy	V11	ns	ns	ns	ns	ns	ns
	V13	ns	-0.88	ns	ns	ns	ns
	R1	ns	-0.96	ns	ns	ns	-0.86
	R2	ns	-0.95	ns	ns	ns	-0.87
	R3	ns	-0.97	ns	ns	ns	-0.90
	R4	ns	-0.94	ns	ns	ns	-0.87
	R5	ns	-0.87	ns	ns	ns	ns

* ns = not significant at the 1% level of probability.

Table 2.8 Area under the curve of crop growth and rust progress in lentil at Akaki, Ethiopia

Treatment	Leaf Area Index (LAI)	Incidence	Severity		
			Upper canopy	Middle canopy	Lower canopy
0	47	1371	950	2309	2304
1	45	590	547	1152	1242
2	41	476	453	940	1233
3	48	555	223	480	710
4	51	200	100	212	298
LSD.05	NS	144.8	103.6	252.8	263
S.E. (±)		120	86	210	218
C.V. (%)	15.7	18.8	17	19	17

Table 2.9 Regression coefficients or slopes (*b* values) for disease progress curves or apparent infection rates and time required for *Uromyces viciae-fabae* severity to reach 10% (T_{10}) on lentil cultivar EL-142 at Akaki, Ethiopia

Spray frequency	Slope of logit line (b)[a] or apparent infection rate	95% C.I. for b	T_{10} (days)[b]	R^2 (% variability explained by regression)	F-value for regression	t-value for b (regression coefficient)
0	0.118 a ± 0.011	0.026	48 c	0.95	127.48**	11.14**
1	0.086 b ±0.006	0.016	55 bc	0.96	240.60**	14.69**
2	0.098 b ±0.007	0.017	59 b	0.96	312.82**	16.30**
3	0.085 b ±0.005	0.013	58 bc	0.97	320.95**	17.15**
4	0.050 c ±0.007	0.021	94 a	0.82	48.0**	6.47**

[a] b is the linear regression coefficient of logit severity against time. It represents apparent infection rate. Each value is the mean of six replications. Logit $x = \log_e[x/(1-x)]$, where x = the proportion of leaf area affected by rust.

[b] the number of days was calculated by solving the regression equation $\log_e[x/(1-x)] = a + bT$, using values of a and b determined from logit analysis (Zadoks and Schein, 1984) of disease progress curves and substituting x with 0.10. Therefore, T (time in days, i.e. T_{10}) = ln{[0.10/(1-0.10)]-a}/b. Each value is the mean of six replications.

Means followed by the same letter are not significantly different from each other at the 5% level of probability of the LSD test.

** Significant at 1% level of probability.

Table 2.10 Effect of rust (*Uromyces viciae-fabae*) on seed yield of lentil at Akaki, Ethiopia

Spray interval (days)	AUDPC (%-disease days)	Seed yield (kg ha^{-1})	Index	Percent change in yield
5	101	1200	100.0	0.0
10	471	1250	104.2	+4.2
15	530	1010	84.2	-15.8
20	587	890	74.2	-25.8
Unsprayed control	1086	730	60.8	-39.2

Table 2.11 Models describing and predicting lentil yield losses due to rust (*Uromyces viciae-fabae*) during the 2001/02 growing season at Akaki, Ethiopia together with the variance ratio (F-value), standard error (SE) and coefficient of determination

Prediction or loss equation[a]	Canopy layer[b]	Crop growth stage[c]	Model type[d]	F-value[e]	Standard error (SE)		t-statistic for slope	R^2
					Slope	Regression line		
YL = 2.22 + 8.39X	UC	R1	CP	13.18*	2.31	8.95	3.63**	0.81
YL = 1.65 + 1.59X	UC	R1	ADPC	13.62*	0.43	8.83	3.69*	0.82
YL = 1.85 + 0.94X	UC	R2	ADPC	11.82*	0.27	9.35	3.44*	0.80

[a] YL, percentage yield loss; Maximum possible yield loss = 41.7% [YL = 2.22 + 8.39(4.7)].
[b] UC, upper canopy layer.
[c] R1, first bloom; R2, full bloom.
[d] CP, critical-point model that used rust severity measurement at the crop growth stage and canopy layer specified in the Table; ADPC, area under the disease progress curve (% of disease days) model that used the calculated area under the curve truncated at R1crop growth stage.
[e] * and ** significant at the 5% and 1% level of significance, respectively.
Number of observations used to compute the regression (N) = 30 (5 treatments by 6 replications).

Table 2.12 Relationship between yield and yield components of lentil as dependent variables (*y*) and rust severity in three canopy layers on different days after sowing or physiological time/growth stage, as independent variables (*x*)

y[a]	Independent variable (*x*)				Regression model output[e]	
	Layer	DAS[b]	Growth stage[c]	Rust parameter[d]	Equation	R^2
SY	Upper	51	R1	S	$y = 117 - 10.10x$	0.81
			R1	AUDPC	$y = 118 - 1.89x$	0.82
		58	R2	AUDPC	$y = 117 - 0.56x$	0.78
		65	R3	S	$y = 120.4 - 2.1x$	0.73
SYPP	Middle	65	R3	S	$y = 2.87 - 0.01x$	0.78
	Mean of 3 layers	65	R3	S	$y = 2.91 - 0.02x$	0.73
SM	Upper	51	R1	S	$y = 25.1 - 1.14x$	0.77
		65	R3	S	$y = 25.8 - 0.28x$	0.95
		72	R4	S	$y = 27.3 - 0.18x$	0.87
	Middle	51	R1	S	$y = 27.6 - 0.55x$	0.97
		58	R2	S	$y = 26.6 - 0.24x$	0.94
		65	R3	S	$y = 25.5 - 0.08x$	0.89
		72	R4	S	$y = 26.2 - 0.08x$	0.88
		79	R5	S	$y = 27.2 - 0.07x$	0.82
	Lower	44	V13	S	$y = 27.3 - 0.51x$	0.78
		51	R1	S	$y = 27.0 - 0.30x$	0.93
		58	R2	S	$y = 26.5 - 0.15x$	0.90
		65	R3	S	$y = 26.6 - 0.12x$	0.94
		72	R4	S	$y = 27.3 - 0.10x$	0.89
		79	R5	S	$y = 27.8 - 0.08x$	0.76
	Mean of 3 layers	44	V13	S	$y = 27.1 - 0.85x$	0.88
		51	R1	S	$y = 26.5 - 0.48x$	0.95
		58	R2	S	$y = 26.4 - 0.24x$	0.93
		65	R3	S	$y = 26.0 - 0.12x$	0.93
		72	R4	S	$y = 26.9 - 0.12x$	0.89
		79	R5	S	$y = 27.5 - 0.09x$	0.82
	Upper	86 (Complete)	R6	AUDPC	$y = 27.1 - 0.01x$	0.90
	Middle	86 (Complete)	R6	AUDPC	$y = 26.7 - 0.003x$	0.89
	Lower	86 (Complete)	R6	AUDPC	$y = 27.4 - 0.003x$	0.88
	Mean of 3 layers	86 (Complete)	R6	AUDPC	$y = 27.1 - 0.004x$	0.89
	Upper	58, 65	R2, R3	S	$y = 26.0 + 0.21x_1 - 0.12x_2$	0.98

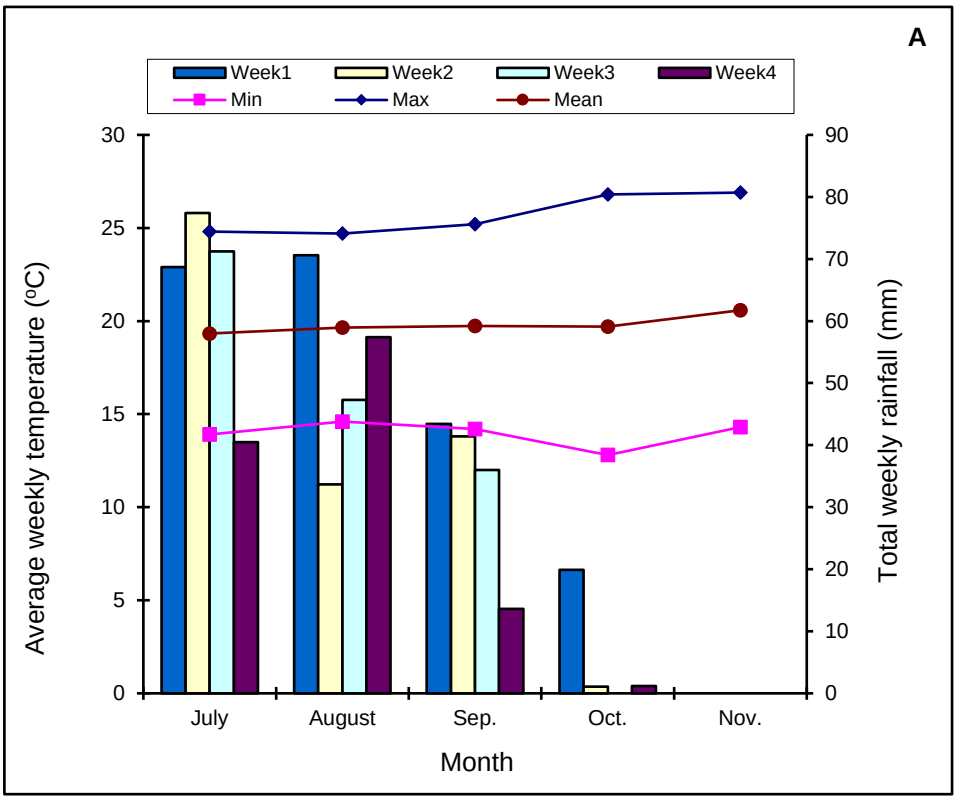
[a] SY, seed yield m^{-2}; SYPP, seed yield per plant (g); SM, seed mass (mg).
[b] DAS, days after sowing.
[c] R1, first bloom; R2, full bloom; R3, early pod; R4, flat pod; R5, full seed; R6, full pod cavity.
[d] S, severity; AUDPC, area under disease progress curve (% disease days).
[e] For each yield and yield component/canopy layer/time/growth stage combinations each, the model using *x* as independent variable giving the highest R^2 value, with significance of the regression equation and of every regression coefficient < 0.05, is shown. The number of observations used to compute the regressions was 30 (5 treatments by 6 replications).

Table 2.13 Relationship between days to maturity of lentil as dependent variables (Y) and rust severity in three canopy layers on different days after sowing or physiological time/growth stage, as independent variables (X)

	Independent variable (X)			Regression model output	
Y	Canopy layer	Days after sowing	Growth stage	Equation	R^2
Days to maturity	Upper	51	R1	Y = 115.92 - 3.020X	0.84
		58	R2	Y = 116.24 - 1.212X	0.90
		65	R3	Y = 117.34 - 0.682X	0.89
		72	R4	Y = 120.71 - 0.441X	0.77
	Middle	51	R1	Y = 121.51 - 1.338X	0.87
		58	R2	Y = 119.05 - 0.575X	0.85
		65	R3	Y = 116.66 - 0.197X	0.84
		72	R4	Y = 118.22 - 0.182X	0.80
	Lower	51	R1	Y = 119.65 - 0.693X	0.75
		58	R2	Y = 118.55 - 0.346X	0.75
		65	R3	Y = 119.07 - 0.287X	0.82
		72	R4	Y = 120.63 - 0.239X	0.76

Figure 2.1 Climato-diagrams showing (A) weekly average minimum, mean and maximum temperatures (line graph), and rainfall totals (bar graph) and (B) monthly mean relative humidity and solar adiation in Akaki, Ethiopia during the 2001/2002 cropping season.

(Source: National Meteorological Services Agency, Ethiopia).


A
Week1
Week2
Week3
Week4
Min
Max
Mean
Average weekly temperature (ºC)
Total weekly rainfall (mm)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80
90
July
August
Sep.
Oct.
Nov.
Month

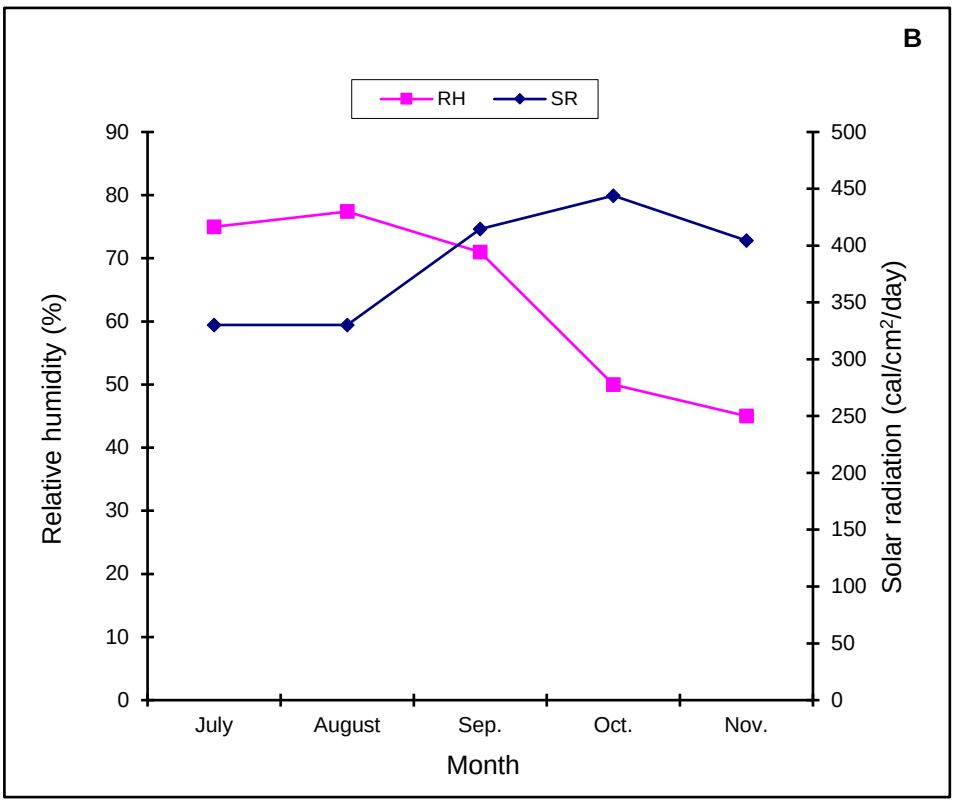

B
RH
SR
Relative humidity (%)
Solar radiation (cal/cm2/day)
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250
300
350
400
450
500
July
August
Sep.
Oct.
Nov.
Month

Figure 2.2 Vertical distribution of rust severity in the unsprayed treatment or plots at different growth stages (GS) of the crop. 0, lower canopy; 1, middle canopy; and 2, upper canopy layer.

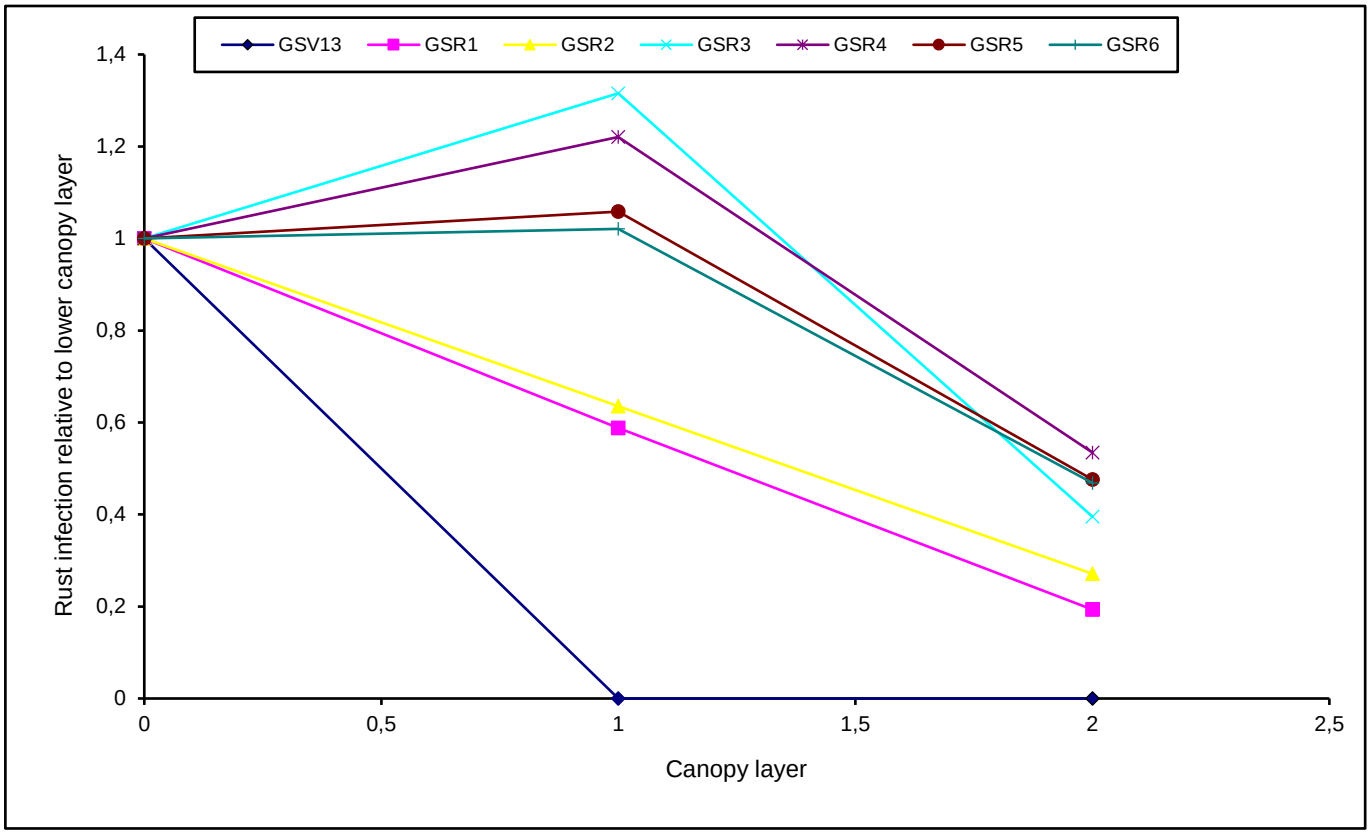
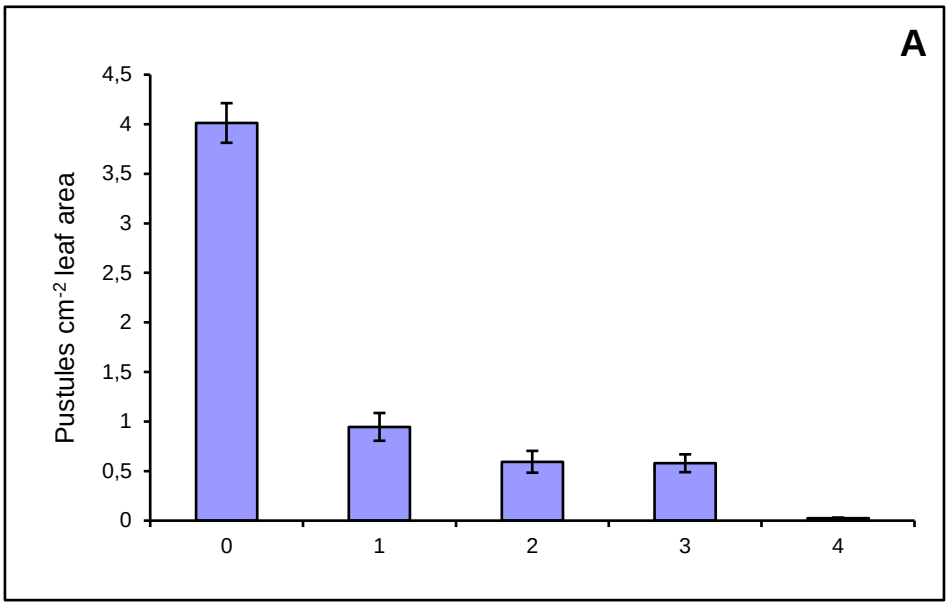

GSV13
GSR1
GSR2
GSR3
GSR4
GSR5
GSR6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
Rust infection relative to lower canopy layer
0
0,5
1
1,5
2
2,5
Canopy layer

Figure 2.3 Effect of spray treatments on (A) pustule density and (B) pustule size. Each data series is the mean of upper, middle and lower canopy layers. Each bar is the mean of six replications and eight assessment dates. Error bars indicate standard deviations. 0, unsprayed control; 1, sprayed every 20 days; 2, sprayed every 15 days; 3, sprayed every 10 days; and 4, sprayed every five days.


A
Pustules cm-2 leaf area
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0
1
2
3
4

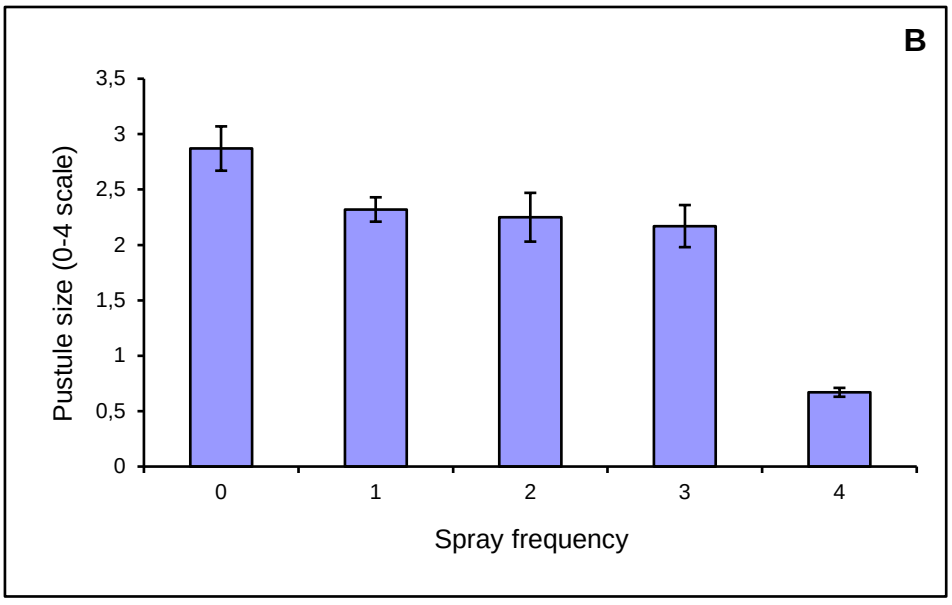
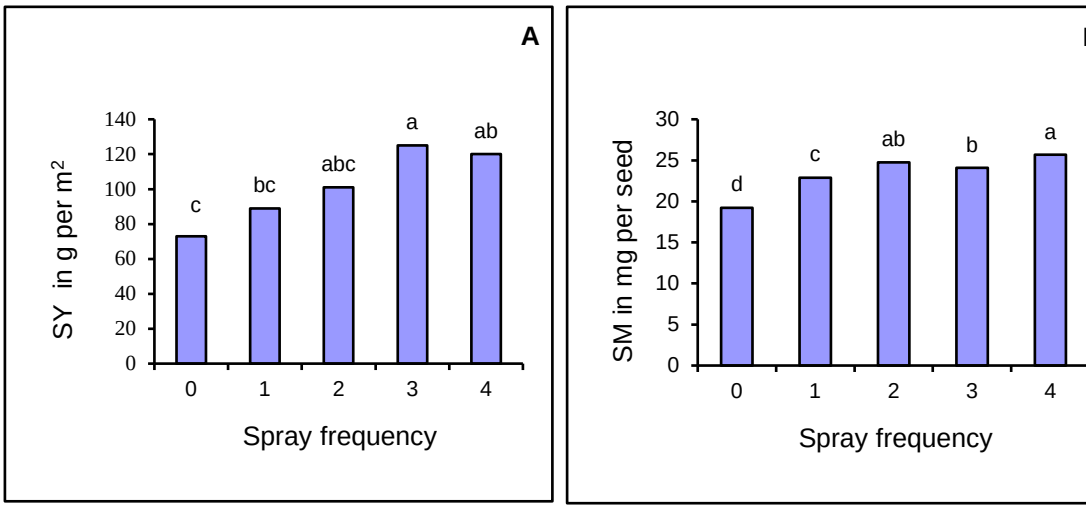
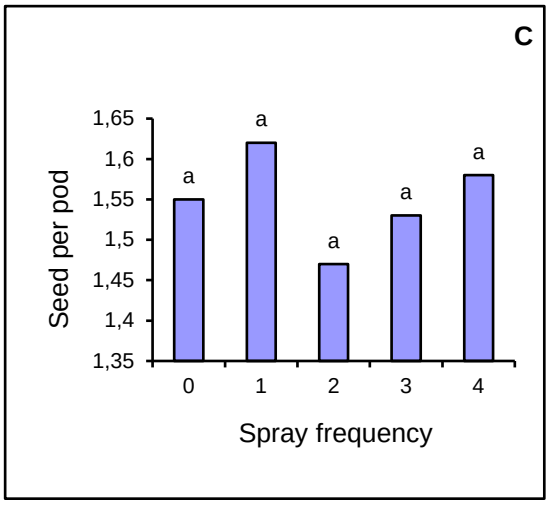
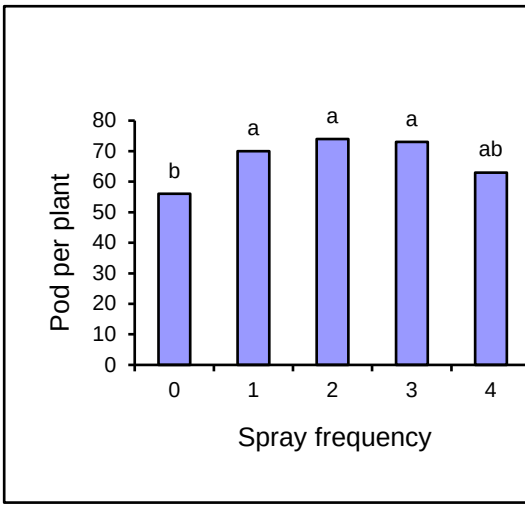

B
Pustule size (0-4 scale)
3,5
3
2,5
2
1,5
1
0,5
0
0
1
2
3
4
Spray frequency

Figure 2.4 Effect of fungicide spray treatments on yield parameters of lentil cv. EL-142 (A) seed yield (SY) in g per m^2, (B) seed mass (SM) in mg per seed, (C) number of seeds per plant, (D) number of pods per plant. Each column is the mean of six replications. Bars denoted by the same letter are not significantly different from each other at the 5% level of significance of the LSD test. 0, unsprayed control; 1, sprayed every 20 days; 2, sprayed every 15 days; 3, sprayed every 10 days; and 4, sprayed every five days.


A
SY in g per m²
140
120
100
80
60
40
20
0
c
bc
abc
a
ab
0
1
2
3
4
Spray frequency

SM in mg per seed
30
25
20
15
10
5
0
d
c
ab
b
a
0
1
2
3
4
Spray frequency


C
Seed per pod
1,65
1,6
1,55
1,5
1,45
1,4
1,35
a
a
a
a
a
0
1
2
3
4
Spray frequency


Pod per plant
80
70
60
50
40
30
20
10
0
b
a
a
a
ab
0
1
2
3
4
Spray frequency

B

D

Figure 2.5 Yield components of lentil as percent of the reference treatment (treatment with minimum rust infection, i.e. treatment 4). SY, seed yield; SM, seed mass; PP, pods per plant; SP, seeds per pod. Bars per item represent spray frequency from 0 (unsprayed control) to 4 (sprayed every five days). Each bar is a mean of six replications.

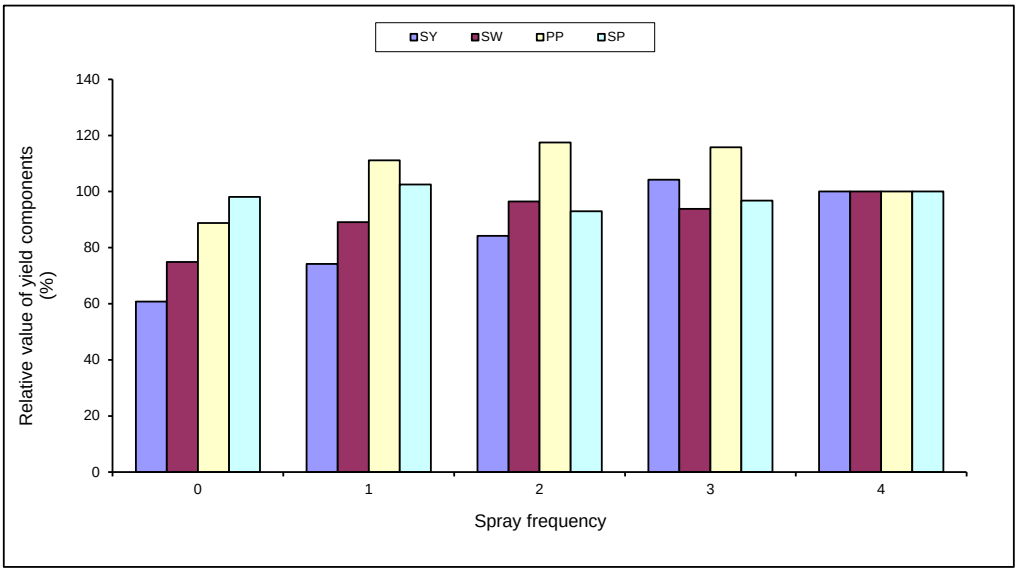
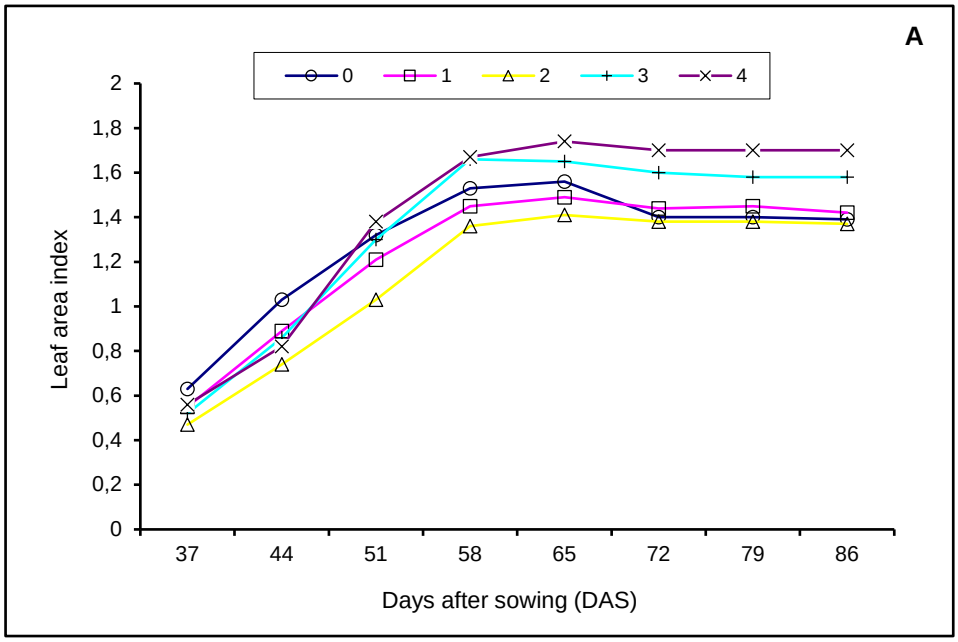

SY
SW
PP
SP
Relative value of yield components (%)
140
120
100
80
60
40
20
0
0
1
2
3
4
Spray frequency

Figure 2.6 (A) Lentil crop growth progress curves, expressed as leaf area index (LAI) against time in days from sowing and (B) area under the crop growth curves (AUCGC) of lentil cv. EL-142. I-bars represent the standard deviations of the means. 0, unsprayed control check; 1, sprayed every 20 days; 2, sprayed every 15 days; 3, sprayed every 10 days; and 4, sprayed every 5 days.


A
0
1
2
3
4
Leaf area index
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
37
44
51
58
65
72
79
86
Days after sowing (DAS)

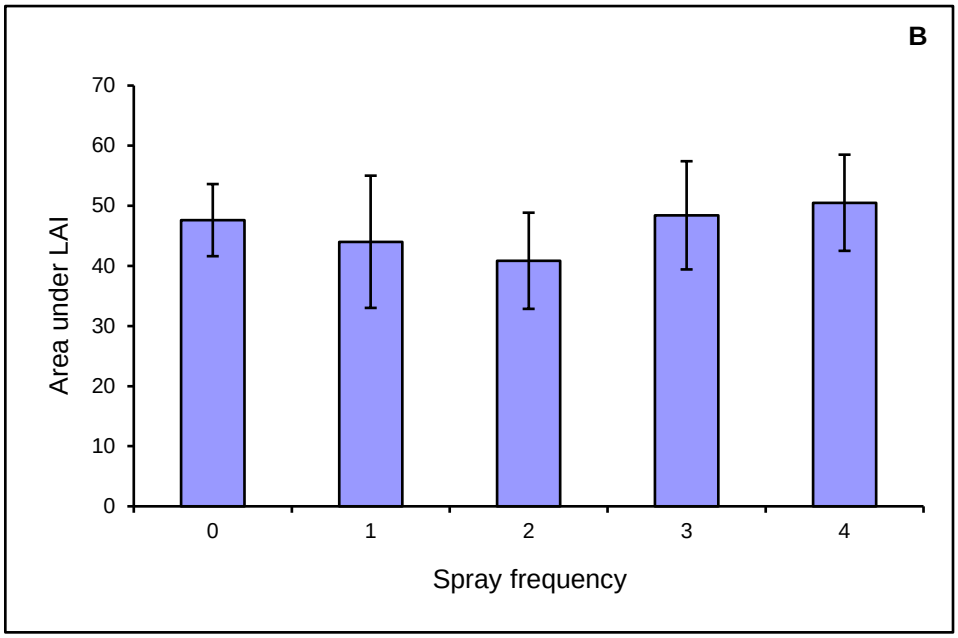
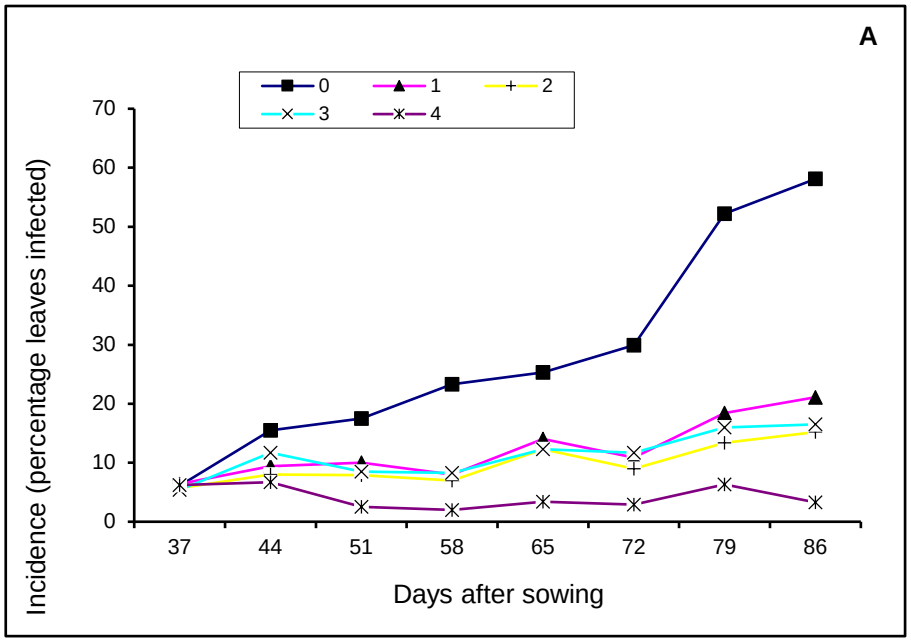

B
Area under LAI
70
60
50
40
30
20
10
0
0
1
2
3
4
Spray frequency

Figure 2.7 (A) Rust incidence progress curves and (B) rust severity progress curves on lentil cv. EL-142 at Akaki. Rust severities are means of 216 data points (12 sample plants x 3 canopy layers x 6 replications). 0, unsprayed control check; 1, sprayed every 20 days; 2, sprayed every 15 days; 3, sprayed every 10 days; and 4, sprayed every 5 days.


A
0
1
2
3
4
70
60
50
40
30
20
10
0
Incidence (percentage leaves infected)
37
44
51
58
65
72
79
86
Days after sowing

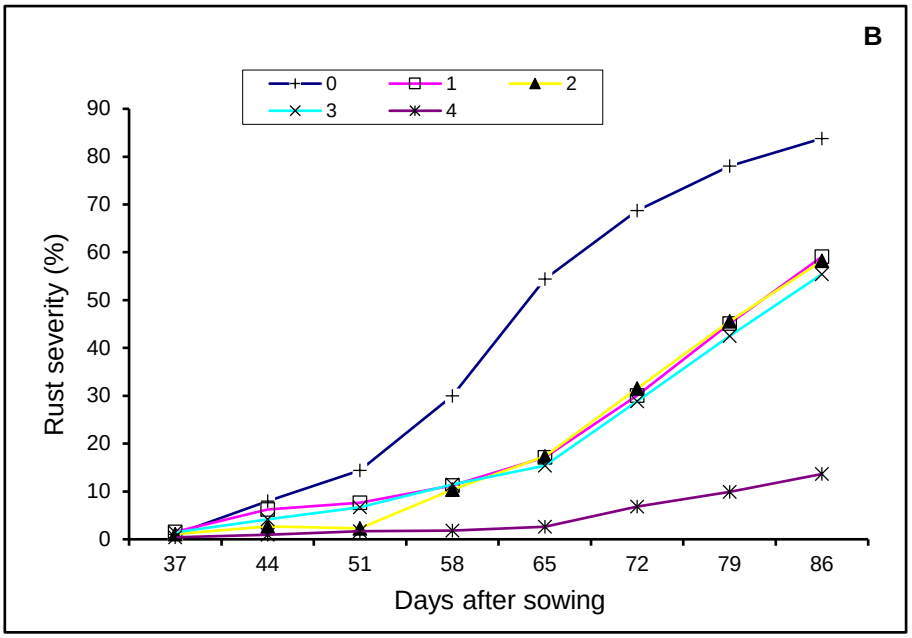

B
0
1
2
3
4
90
80
70
60
50
40
30
20
10
0
Rust severity (%)
37
44
51
58
65
72
79
86
Days after sowing

Figure 2.8 Rust progress curves in a susceptible lentil variety, EL-142. (A) unsprayed control, (B) sprayed every 20 days, (C) sprayed every 15 days, (D) sprayed every 10 days, and (E) sprayed every five days. UC, upper canopy; MC, middle canopy; and LC, lower canopy layer.

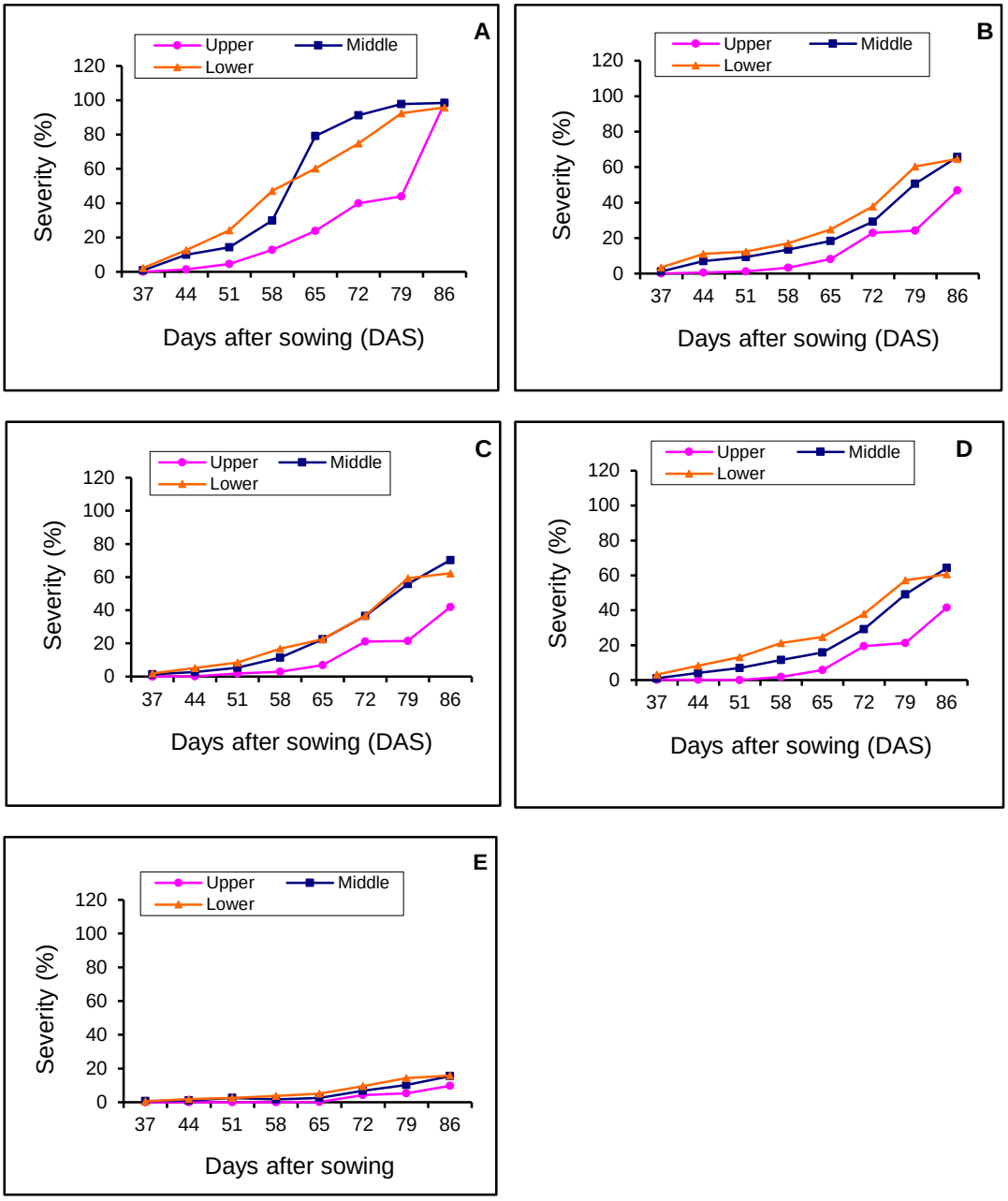

A
Upper
Middle
Lower
Severity (%)
120
100
80
60
40
20
0
37 44 51 58 65 72 79 86
Days after sowing (DAS)
B
Upper
Middle
Lower
Severity (%)
120
100
80
60
40
20
0
37 44 51 58 65 72 79 86
Days after sowing (DAS)
C
Upper
Middle
Lower
Severity (%)
120
100
80
60
40
20
0
37 44 51 58 65 72 79 86
Days after sowing (DAS)
D
Upper
Middle
Lower
Severity (%)
120
100
80
60
40
20
0
37 44 51 58 65 72 79 86
Days after sowing (DAS)
E
Upper
Middle
Lower
Severity (%)
120
100
80
60
40
20
0
37 44 51 58 65 72 79 86
Days after sowing

Figure 2.9 Regression lines fitted to logit severity data of each treatment averaged over six replications. Logit severity was determined using the transformation $\log_e[x/(1-x)]$, where x = proportion of leaf area diseased. 0, unsprayed control check; 1, sprayed every 20 days; 2, sprayed every 15 days; 3, sprayed every 10 days; and 4, sprayed every five days.

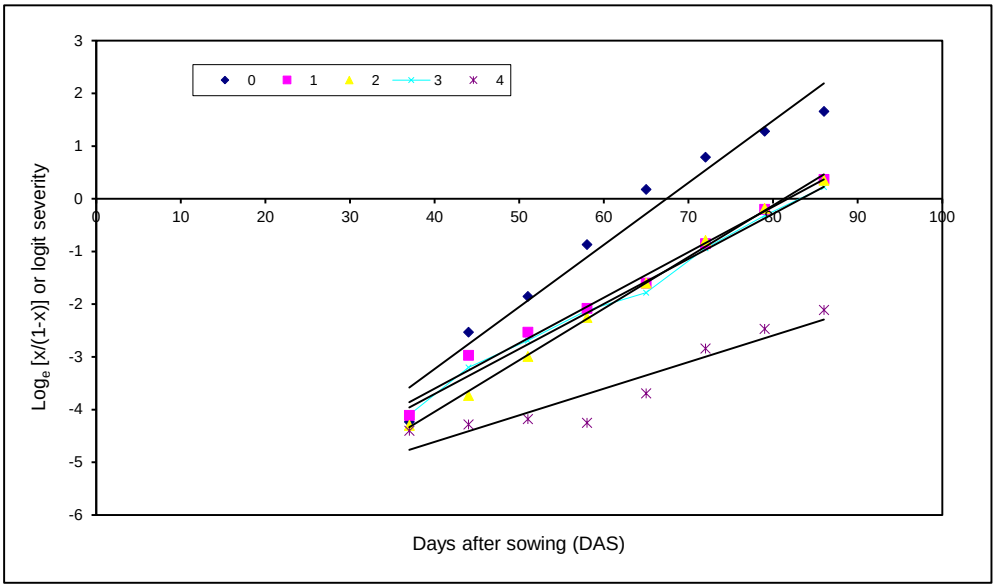
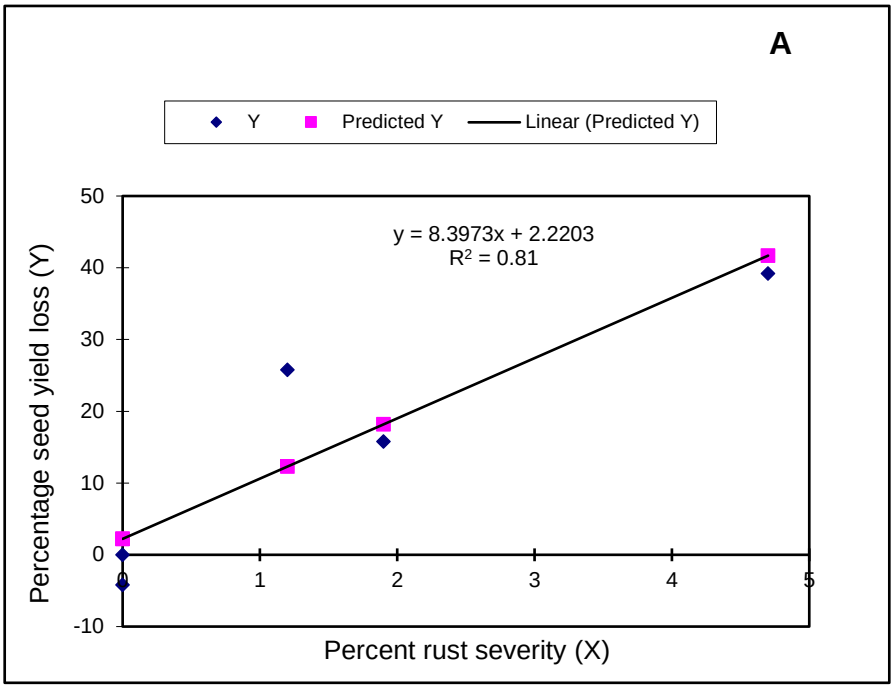

0
1
2
3
4
3
2
1
0
-1
-2
-3
-4
-5
-6
0
10
20
30
40
50
60
70
80
90
100
Loge [x/(1-x)] or logit severity
Days after sowing (DAS)

Figure 2.10 Relationship between yield loss in lentil and rust severity. (A) regression of yield loss of lentil cv. EL-142 on rust severity in the upper canopy at R1 growth stage, and (B) distribution pattern of a plot of residuals versus predicted yield loss of lentil due to rust in the upper canopy layer at R1 growth stage.


A
Y
Predicted Y
Linear (Predicted Y)
y = 8.3973x + 2.2203
R² = 0.81
Percentage seed yield loss (Y)
Percent rust severity (X)
50
40
30
20
10
0
-10
0
1
2
3
4
5

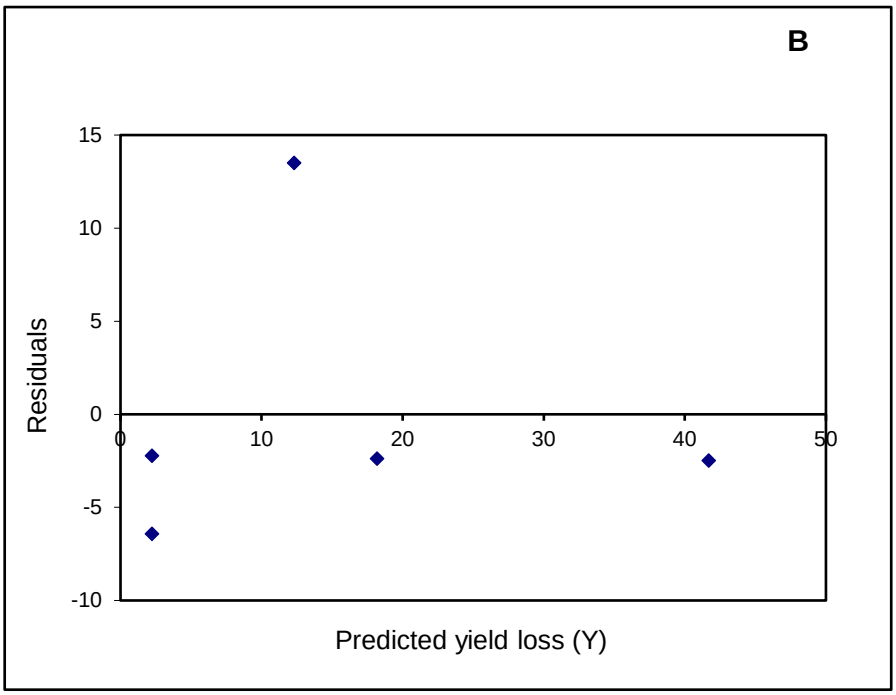
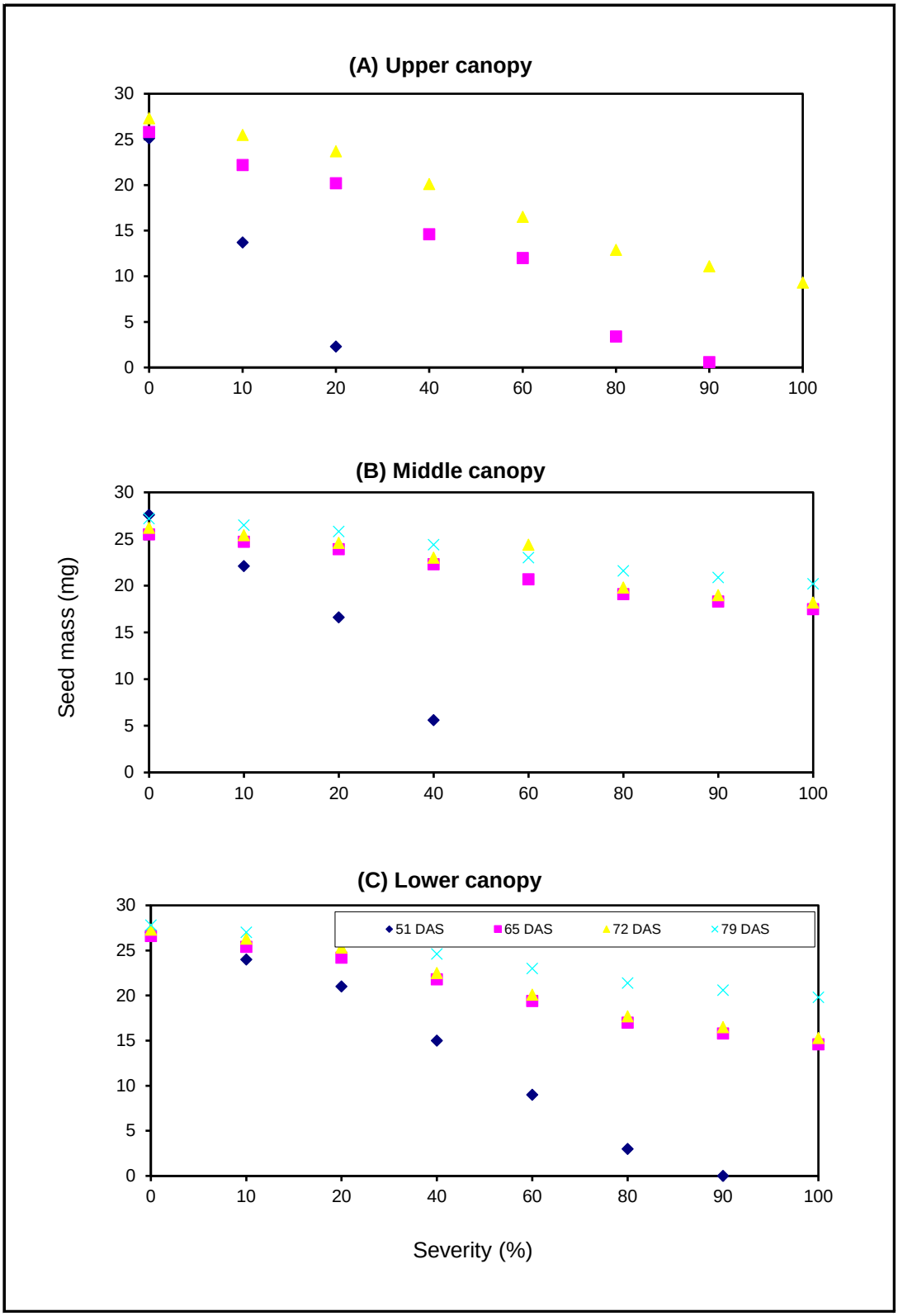

B
Residuals
Predicted yield loss (Y)
15
10
5
0
-5
-10
0
10
20
30
40
50

Figure 2.11 Relationship between rust severity and seed mass at different days after sowing in the (A) upper, (B) middle and (C) lower canopy layers. Seed mass was determined by averaging mass of five seed lots of 100 seeds each and dividing by 100. The fitted curves correspond to the models described in Table 2.12.


(A) Upper canopy
30
25
20
15
10
5
0
0
10
20
40
60
80
90
100
(B) Middle canopy
Seed mass (mg)
30
25
20
15
10
5
0
0
10
20
40
60
80
90
100
(C) Lower canopy
51 DAS
65 DAS
72 DAS
79 DAS
30
25
20
15
10
5
0
0
10
20
40
60
80
90
100
Severity (%)

Appedix 2.1 Action Threshold

- LSD or critical difference (CD) between two treatment means at 5% level of probability = 33.8 g m^2
- Yield of treatment 4 = 120 g m^2
- Severity record for treatment 0 (no spray or treatment with maximum disease) = 4.7% in the upper canopy layer at R1 growth stage
- Yield of treatment 0 = 73 g m^2
- Difference between treatment 4 and 0 = 120 – 73 g m^2 = 47 g m^2
- The above yield difference between the two treatments is 39% in terms of percent yield loss
- Yield loss (reduction) for every 1% disease severity = 8.39% (YL = 8.39X)
- Solving the equation 39% = 8.39X will give 4.65%
- From the above arguments, the severity 4.65% in the upper canopy layer at R1 growth stage could then be taken as the disease level that result in a critical or significant yield loss difference between two rust control treatments
- Therefore, the observed 4.7%-severity level (point) could be taken as action threshold level

CHAPTER 3

THE EFFECT OF RUST ON DRY MATTER DEGRADABILITY, NITROGEN, PHOSPHORUS AND CRUDE PROTEIN CONTENT OF LENTIL

"We live in a world of sad scientific ironies and economic enigmas. The regions where man first settled down to cultivate plants and thereby initiated what we call 'agriculture' are also the regions which contain the greatest number of hungry people today."

M.S. Swaminathan

ABSTRACT

Lentil (*Lens culinaris* Medik.) straw is a valuable animal feed supplement because of its high protein and carbohydrate content. In Ethiopia, a significant amount is fed to livestock whether it is infected by rust (*Uromyces viciae-fabae*) or not. The dry matter (feed stuff) consumed by the animals should be degradable as this is crucial in determining the availability of nitrogen to microorganisms and amino acids in the small intestine to the host animals. It was, therefore, important to determine the effect of rust on straw degradability. A trial, with rumens of Zebu cows, was conducted to evaluate the degradability of straws having different levels of rust attack. An exponential model was fitted to calculate *in vivo* degradability of dry matter. Following 6 h of incubation, samples of all treatments lost ≥ 40% of the total dry matter, degradation reaching ca. 65% at 72 h. Rust did not significantly ($P \geq 0.05$) decrease degradability of dry matter in the rumen. However, the wash value of dry matter of straw from maximum rust infection was significantly ($P \leq 0.05$) higher compared to the others tested in this study. Little is known about the effect of rust on nitrogen (N), phosphorus (P) and crude protein (CP) content of lentil. Hence, chemical analysis was undertaken to assess these constituents in straw and seed of lentil plants from five treatments with varying degrees of the disease. Straw from plants with maximum rust infection had significantly ($P \leq 0.05$) higher N, P and CP contents than straw from healthy plants. The seed P content of healthy plants tended to be significantly ($P \leq 0.05$) higher than that from rust infected plants. The rust had no significant ($P \geq 0.05$) effect on seed nitrogen content.

INTRODUCTION

Lentils (*Lens culinaris* Medik.) are among the major pulse crops grown in the world (FAO, 2004). The seeds are primarily used for human consumption and serve as an important protein source in countries such as Ethiopia in Africa (Nygaard and Hawtin, 1981). Its straw and pod walls have, furthermore, a high feed value for animals. Lentil production, however, is constrained by a number of diseases. Of these, rust is considered to be the major disease in Ethiopia and Morocco in Africa and India and Bangladesh in Asia (Erskine *et al*., 1994).

Apart from the observations of Reddy and Khare (1984) on elemental and some biochemical differences between rust resistant and susceptible genotypes, information is lacking on the effect of rust attack on seed and straw elemental and biochemical contents. Therefore, information is needed to better understand the changes occurring in rust infected lentil plants, and the present study was undertaken to provide information in this regard. Since dry matter digestibility may also be important in determining the suitability of lentil straw as animal feed, this was also studied.

MATERIALS AND METHODS

Lentil cultivar EL-142 was grown in the field at Akaki, Ethiopia in a randomized complete block design with five treatments and six replications; each replication contained five plots of 3 x 3 m plot size each. The five treatments represented different levels of rust epidemics. The different epidemics were created in the same way as in the yield loss experiment (Chapter 2 of this thesis).

Disease assessment

One week after inoculation and weekly thereafter, rust severity was assessed on each plot following the procedure used in Chapter 2 of this thesis.

Dry matter harvesting

At maturity, 1 m^2 of lentil plants was harvested by uprooting whole plants from the five treatments and their replicates separately. The plants were sun dried for one week daily between 08:00 and 17:00. After this drying period, seeds were removed from the pods of each plant per plot.

Sample preparation and chemical analysis

Immediately after threshing, 20 g straw and 20 g seed per plot were sampled for nitrogen (N) and phosphorus (P) analyses. Analyses of nitrogen and phosphorus contents of sample plants were performed according to the methods of Cottenie (1980) at the Soil and Water Laboratory, Debre Zeit Agricultural Research Center, Ethiopia. Total percentage crude protein (CP) was calculated by multiplying the N content of each sample by 6.25 (Nickerson and Ronsivalli, 1978; McDonald *et al*., 1995).

Sample preparation for degradability evaluation

After threshing, representative straw samples were taken from each treatment, dried at 60°C overnight and ground into 2 mm particle size using a Willey mill and stored until analysis. Degradability was evaluated at the Animal Nutrition Laboratory, Debre Zeit Agricultural Research Center, Ethiopia. Samples from all the treatments and their replicates were

individually analyzed for dry matter according to the procedure of the Association of Official Analytical Chemists (AOAC, 1984). Degradability was determined using the Nylon Bag method as described by Orskov and McDonald (1979). Samples (2.5 g) of the 2 mm sieve size were placed in nylon bag of 6 x 12 cm and 41 μm pore size and incubated for 6, 12, 24, 48 and 72 h in the rumen of five fistulated Zebu oxen. Every sample was incubated in triplicate for each incubation hour and placed in the rumen of each fistulated ox starting with the 72 h and adding the others in descending order. After six hours of the last sample placement all bags were taken out at the same time, washed thoroughly, dried, weighed and dry matter disappearance was described by the exponential model (Orskov and McDonald, 1979):

$$P = a + b\,[1-e^{-ct}]$$

where P is the potential dry matter disappearance at time t, a is the rapidly soluble fraction, b is the potentially degradable fraction and c is the rate of degradation.

Lag time (TL) was estimated as

$$TL = (-1/c)\log\{1-[(W-a)/b]\}$$

where c is the rate of degradation, W is washing value at 0 h, a is the soluble fraction and b is the potentially degradable fraction.

Data analyses

Analysis of variance (ANOVA) was computed using an MSTAT-C statistical software (Bricker, 1991). Least significant differences (LSD) were calculated to test was used for

compare treatment means. Regressions of N, CP, and P with disease at the different crop growth stage and canopy layers were also computed.

RESULTS

Seed and straw nitrogen (N) and total crude protein (TCP) contents

The straw from rust infected plants had significantly ($P \leq 0.05$) higher N and CP contents when compared to rust free plants (Table 3.1). The straw N and CP were 67 and 60 %, respectively, higher in rust infected plants than in rust free plants (Figs. 3.1A and 3.1B). Contrary to the N and CP contents of the straw, seed N and CP contents did not differ significantly ($P > 0.05$) between the treatments.

Seed and straw phosphorus (P) content

Differences in phosphorus content of the seeds were highly significant ($P \leq 0.01$) among treatments. P content of seeds from the rust free treatment was the highest. The percentages P in seeds from all fungicide treated plots were significantly higher than in seeds from the unsprayed treatment where the rust was allowed to take its full course of development. Treatment differences in P content of the straw were significant ($P \leq 0.05$), as well (Table 3.1).

The straw P content was 0.28% (280 mg/100 g straw) and 0.50% (500 mg/100 g straw) in healthy and diseased plants, respectively; while for the seeds, this was 0.60% and 0.39% (Table 3.1). The disease significantly ($P \leq 0.05$) decreased the seed P content, by more than 30% in the unsprayed control treatment as compared to the rust-free treatment (treatment 4) The straw P content, however, was higher in plants with maximum disease severity by about 78% when compared with that in rust free plants (Table 3.2).

Relationship between rust severity, N, P and CP

Significant relationships between rust disease at different crop growth stages and canopy layers and elemental/biochemical constituents are shown in Table 3.3. Rust at R1 growth stage in the upper canopy layer significantly affected N, P and CP contents. There was 0.14% and 0.87% increase in N and CP, respectively, for every 1% rust severity in lentil straw. Straw N was positively related to rust severity in all the canopies mainly between R1 and R5 crop growth stage (R^2 = 0.73 to 0.92. Similarly, straw CP was positively related to the rust severity (R^2 = 0.75 to 0.92).

Straw P content was positively related to the rust disease in all the canopy layers at different crop growth stages (R^2 = 0.73 to 0.95). In contrast, seed P was negatively related to rust severity at the crop growth stages R4 to R6 in the upper canopy layer (R^2 = 0.79 to 0.85), R1 to R6 in the middle canopy layer (R^2 = 0.72 to 0.83), and R4 to R5 in the lower canopy layer (R^2 = 0.73 to 0.83).

Dry matter disappearance

No significant differences for dry matter breakdown were obtained between treatments at any of the incubation periods. However, disease severity had an effect on the washing value (0 h incubation period). The straw from lentil plants with high rust infection had a significantly ($P \leq 0.05$) higher washing value than the straw from minimum rust infection (Table 3.4).

DISCUSSION

In general, lentil seeds are rich in protein, with protein content varying from 23.4 to 36.4% (Hawtin *et al*., 1977). Concentration of straw protein ranges from 4.2 to 10.6% (Singh and Singh, 1997). In present study, the crude protein content of straw including pod walls, leaflets and branches ranged from 7.6 to 12.3%, with the highest being recorded in rust infected plants. Rust infection increased protein content in straw (stem, leaves branches and pod walls). This increase was probably due to the increased protein production in response to rust attack. A positive correlation between rust severity and protein content of straw supports this point of view. The concentration of protein in rust infected plants, however, depended on the crop growth stage and leaf canopy layer. Rust attack in the upper canopy layer during early flowering (R1) triggered more protein production as indicated by the high slope value (0.87) of the regression line (Table 3.5).

In most cases, higher plants are known to produce a large variety of proteins, both with known and unknown functions, in response to pathogen attack or environmental stress (Bowles, 1990; Kombrink and Somssich, 1997; Van Loon and Van Strien, 1999; Kombrink and Schmelzer, 2001). In addition, high levels of N and total protein are produced in wheat resulting from infection by the fungus *Chaetomium globosum* Kunze (Biswas *et al*., 2002). Crude protein content of winter wheat (cv. Avalon) declined following control of *Septoria tritici* Roberge in Desm. [*Mycosphaerella graminicola* (Fuckel) J. Shröt] (Gooding *et al*., 1994). However, the type and function of the excess protein in rust infected plants recorded in the present study are not clear.

In contrast to N, which was higher in infected plants, the present study showed that the P content of seed was significantly less in diseased plants. This phenomenon was probably a

result of interference by the pathogen of mobilization of P from vegetative parts of the plant. This was evident from the negative and positive correlation of P content of seed and straw, respectively. Translocation of assimilates (assimilate partitioning) is influenced by a stress factor and development stage of stress experiencing plants (Aggarawal and Sinha, 1984). The seed P level in rust-infected plants was 0.39% (390 mg/100 g dry seed). This level is below the standard seed P level reported for lentils, i.e. about 0.522% (522 mg/100 g dry seed) (Abu Shakra and Tannous, 1981).

Phosphorus is crucial for energy transfer and formation of sugars (Dubey, 2001). The element is known to play a vital role in energy metabolism in the formation of sugar phosphates and adenosine di- and tri-phosphates (ADP and ATP). The latter is an energy rich compound that may be used to enhance biochemical reactions during the performance of essential life processes (McDonald *et al*., 1995). The lower seed P content, thus, implies a decreased level of ADP and ATP in seeds.

The digestibility trial resulted in non-significant differences between treatments for the dry matter disappearance at all incubation periods. The degradability of lentil straws tested in the present study falls within the range reported for rumen degradability of pulse straw (Anonymous, 2000). Therefore, the straw from rust-infected plants was as digestible as that from rust free plants and may be suitable for animal consumption. However, feeding rust infected straw to animals might cause some health problems. Evidence exists that fungus contaminated feeds can cause poisoning in animals, for instance rusted wheat plants are less palatable and are sometimes mildly toxic to livestock (Wiese, 1987).

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Table 3.1 Effects of rust (*Uromyces viciae-fabae*) on yield quality of lentil

Spray interval (days)	AUDPC (%-disease days)	Nitrogen content (%)[a]		Crude protein content (%)		Phosphorus content (%)	
		Seed	Straw	Seed	Straw	Seed	Straw
5	203	5.0	1.2	31.0	7.7	0.60	0.28
10	471	5.0	1.3	31.4	8.4	0.53	0.44
15	875	5.0	1.2	31.0	7.6	0.45	0.43
20	980	5.4	1.3	33.7	8.0	0.43	0.45
Unsprayed control	1854	5.4	2.0	33.7	12.3	0.39	0.50
$LSD_{.05}$	158	NS	0.46	NS	2.88	0.12	0.085
CV (%)	14	16	27	16	27	20	15
SE (±)	76	0.82	0.38	5.1	2.4	0.10	0.07

[a] NS = Non-significant.

Table 3.2 Effect of rust (*Uromyces viciae-fabae*) on phosporus (P) content of lentil crop at Akaki, Ethiopia, 2001

Spray interval (days)	AUDPC (% disease days)	Seed			Straw		
		% P	Index	% Change in P content	% P	Index	% Change in P content
5	203	0.60	100	0	0.28	100	0
10	471	0.53	88.3	– 11.7	0.44	157.1	+ 57.1
15	875	0.45	75.0	– 25.0	0.43	153.6	+ 53.6
20	980	0.43	71.7	– 28.3	0.45	160.7	+ 60.7
Control	1854	0.39	65	– 35.0	0.50	178.6	+ 78.6

Table 3.3 Relationship between yield qualities of lentil as dependent variables (*y*) and rust severity in three canopy layers on different physiological time represented by crop growth stage, as independent variables (*x*)

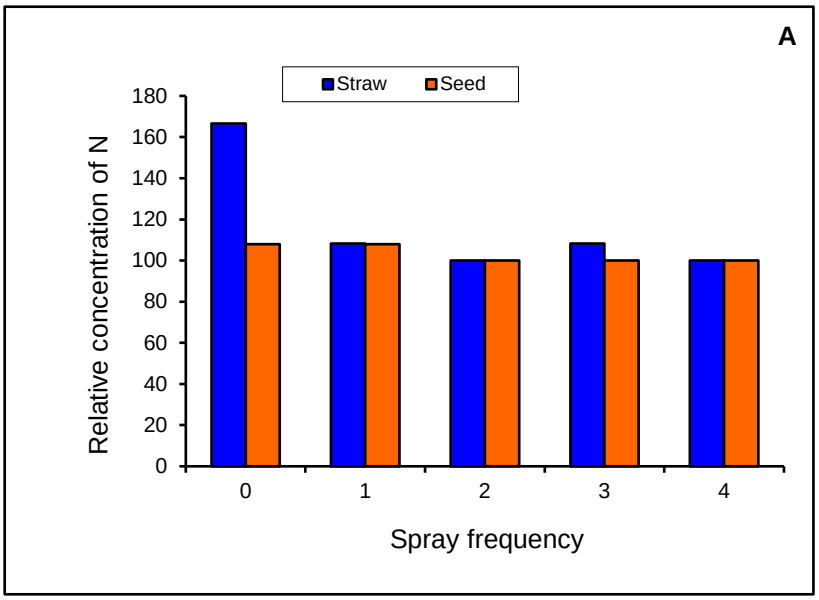
Y	Independent Variable (x)			Regression model output	
	Layer	DAS	Growth Stage	Equation	R^2
% N-Straw	Upper	51	R1	$y = 1.18 + 0.14x$	0.73
		58	R2	$y = 1.15 + 0.061x$	0.92
		65	R3	$y = 1.10 + 0.034x$	0.86
		79	R5	$y = 0.94 + 0.020x$	0.75
	Middle	51	R1	$y = 0.93 + 0.06x$	0.76
		58	R2	$y = 1.02 + 0.028x$	0.83
		65	R3	$y = 1.12 + 0.01x$	0.92
		72	R4	$y = 1.04 + 0.01x$	0.87
	Lower	51	R1	$y = 0.96 + 0.036x$	0.82
		58	R2	$y = 1.01 + 0.019x$	0.88
		65	R3	$y = 1.00 + 0.015x$	0.87
		72	R4	$y = 0.93 + 0.012x$	0.77
% P-Seed	Upper	72	R4	$y = 0.06 - 0.001x$	0.83
		79	R5	$y = 0.06 - 0.001x$	0.79
		86	R6	$y = 0.07 - 0.0001x$	0.85
	Middle	51	R1	$y = 0.06 - 0.002x$	0.72
		58	R2	$y = 0.06 - 0.001x$	0.72
		79	R5	$y = 0.06 - 0.0001x$	0.79
		86	R6	$y = 0.06 - 0.0001x$	0.83
	Lower	72	R4	$y = 0.06 - 0.0001x$	0.73
		79	R5	$y = 0.06 - 0.0001x$	0.83
% P-Straw	Upper	72	R4	$y = 0.03 + 0.001x$	0.86
		79	R5	$y = 0.03 + 0.001x$	0.83
		86	R6	$y = 0.02 + 0.0001x$	0.99
	Middle	51	R1	$y = 0.03 + 0.002x$	0.73
		58	R2	$y = 0.03 + 0.001x$	0.73
		79	R5	$y = 0.03 + 0.0001x$	0.84
		86	R6	$y = 0.02 + 0.0001x$	0.95
	Lower	44	V13	$y = 0.03 + 0.002x$	0.80
		51	R1	$y = 0.03 + 0.001x$	0.76
		72	R4	$y = 0.03 + 0.0001x$	0.81
		79	R5	$y = 0.03 + 0.0001x$	0.94
		86	R6	$y = 0.03 + 0.0001x$	0.95
% Crude Protein-Straw	Upper	51	R1	$y = 7.42 + 0.873x$	0.73
		58	R2	$y = 7.21 + 0.378x$	0.92
		65	R3	$y = 6.89 + 0.210x$	0.89
		79	R5	$y = 5.92 + 0.123x$	0.75
	Middle	51	R1	$y = 5.82 + 0.385x$	0.76
		58	R2	$y = 6.37 + 0.176x$	0.84
		65	R3	$y = 7.02 + 0.063x$	0.92
		72	R4	$y = 6.53 + 0.058x$	0.87
	Lower	51	R1	$y = 6.05 + 0.224x$	0.82
		58	R2	$y = 6.31 + 0.116x$	0.89
		65	R3	$y = 6.27 + 0.091x$	0.87
		72	R4	$y = 5.83 + 0.075x$	0.78
Seed protein yield per unit area	Upper	51	R1	$y = 37.23 - 3.02x$	0.89
		58	R2	$y = 36.95 - 1.07x$	0.78
		65	R3	$y = 37.9 - 0.597x$	0.73

Table 3.4 Effects of rust infection on dry matter degradability and crude protein content of lentil haulm incubated in the rumen of Zebu oxen

Treatment	AUDPC (%-disease days)	Crude protein content (% CP)	Dry matter disappearance						
			Time (hour)*						
			0	6	12	24	48	72	96
0	1854	12.23	37	42	52	59	66	68	66
1	980	8.07	32	42	44	56	63	67	63
2	875	7.55	33	42	48	58	64	67	63
3	471	8.40	33	42	49	52	63	65	62
4	203	7.60	32	40	48	55	63	65	62
$LSD_{.05}$	158	2.73	3.77	NS	NS	NS	NS	NS	NS
SE (±)	76	2.30	7.66	7.66	5.94	6.77	5.79	6.65	5.32
CV (%)	14	26.22	18.45	9.45	12.37	12.13	9.08	10.01	8.43

* NS = non-significant.

Figure 3.1 (A) Relative concentration of N in lentil straw and seed and (B) concentration of crude protein in lentil straw and seed in different fungicide treatments against *Uromyces viciae-fabae*. 0, unsprayed control; 1, sprayed every 20 days; 2, sprayed every 15 days; 3, sprayed every 10 days; and 4, sprayed every 5 days. Treatment 4 was used as reference.


A
Straw
Seed
Relative concentration of N
180
160
140
120
100
80
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20
0
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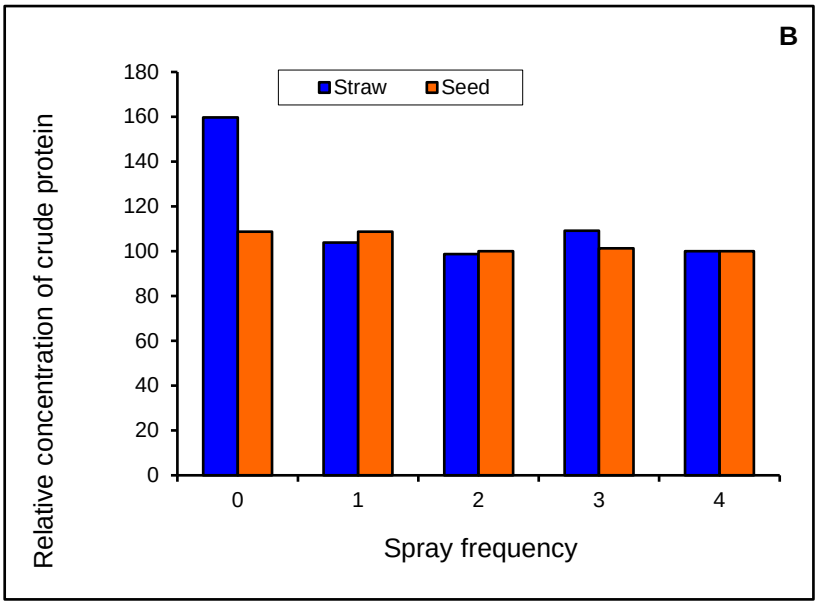
1
2
3
4
Spray frequency


B
Straw
Seed
Relative concentration of crude protein
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
Spray frequency

CHAPTER 4

A SETTLING TOWER FOR QUANTITATIVE DEPOSITION OF UREDINIOSPORES OF *UROMYCES VICIAE-FABAE*

"Nothing is easier than to invent a method of estimation."

Sir R.A. Fisher

ABSTRACT

An inoculation technique resulting in uniform spore deposition for obtaining reproducible and accurate data on host responses is required for quantitative studies of resistance expression, for instance, testing infection efficiency. The feasibility of obtaining uniform spore deposition on *Lens culinaris*, a plant with compound leaves, was investigated using a settling tower. Uniformity of spore deposition on adhesive coated glass slides was assessed by dispersing 1, 2, 4 and 8 mg spores of *Uromyces viciae-fabae*, the causal agent of lentil rust, into the tower. When an 8 mg spore quantity was discharged non-significant ($P > 0.05$) differences were found within and between the different locations on the target area for number of spores deposited per square centimetre. A linear relation was found between mass of spores discharged into the tower and spores deposited. Counts for 1, 2, 4 and 8 mg were, respectively, 88, 280, 570 and 970 spores per square centimetre. Uniformity of spore deposition increased as the amount discharged increased, and standard deviation as percentage of the mean for spore deposition was inversely related to the spore quantity dispersed. When plants of lentil cultivar EL-142 were exposed to spores in the settling tower, differences in numbers of lesions per square centimetre leaf area were non-significant ($P > 0.05$) within and between inoculation runs. Following dispersal of 8 mg urediniospores for a 3 min settling period, a mean of 140 uredinia (standard deviation = 7) occurred per square centimetre leaf area.

INTRODUCTION

Rust caused by *Uromyces viciae-fabae* (Pers.) J. Schröt is one of the most important diseases of lentil (*Lens culinaris* Medik.). The disease causes significant yield losses when rust epidemics occur in lentil-growing countries (Erskine *et al*., 1994). Host resistance has been adopted as the most viable and economical method of lentil rust control worldwide. Screening and selection for rust resistance, therefore, continues to be a priority in lentil breeding programs in countries where rust is a production constraint.

Currently, the standard method followed in resistance screening is exposing breeding lines to natural infection in areas known for regular and severe rust epidemics (Erskine *et al*., 1994). However, it is unwise to rely on field screening alone since weather conditions are not always conducive for rust development. Therefore, field screening needs to be supplemented with glasshouse evaluations. Reproducible and accurate data are requirements in detecting phenotypic differences in host response to the rust. To obtain such measurements and make quantitative comparisons, uniform deposition of spore loads that can be varied intentionally in different pathological experimentations is considered as a pre-requisite (Schein, 1964). However, the relationship between rust inoculum and infection of lentil by urediniospores of *U. viciae-fabae* has not been studied in a quantitative way. Development of a system that will allow consistent discrimination among lentil genotypes in components of rate-reducing resistance will thus be beneficial to breeding programmes.

Settling towers have often been used to study quantitative resistance to rusts in cereal crops (Petersen, 1959, Eyal, Clifford and Cladwell, 1968; Mortensen, Green, and Atkinson, 1979; Aslam and Schwarzbach, 1980) and beans (Schein, 1964). In

these studies leaves were usually orientated horizontally to expose the upper surface of leaf blades to equal inoculum quantities. In contrast to plants with simple leaves, adaxial surfaces of crops with compound leaves are more difficult to expose to settling spores in a similar fashion. This is particularly true for lentil whose leaves are sub-sessile, narrowly obovate to elliptical in shape, and pinnate with one to eight pairs of leaflets (Saxena and Hawtin, 1981). The objective of this study was to determine whether uniform rust infection of lentil could be obtained using a settling tower.

MATERIAL AND METHODS

Description of the instrument

The spore settling tower (Fig. 4.1) consists of a galvanized, metal sheeting cylinder (height = 170 cm, diam. = 50 cm), coated with glossy paint and placed over a turntable at the bottom of the cylinder. The Perspex turntable is rotated by a variable speed electric motor (Groschoppu Co., Viersen/Rhld.). A Perspex lid with a centre aperture covered by a shutter, serving as an entrance point for inoculum release, fits on top of the cylinder.

Spore discharge

Dry spores without any carrier are placed on the short arm of an L-shaped attachment to an air gun, in line with and 5 cm from its muzzle. The gun is carefully inserted through the hole in the lid and held 1.2 m from the top of the surface of the turntable. By releasing the trigger the spores are forcibly dispersed into the settling tower by a jet of air emitted from the barrel. The inoculator is then immediately removed and the lid opening shut. The motor is switched off after a specified time of spore deposition and the target units (slides, agar plates or plants) are removed without delay.

In vitro deposition assay

Uniformity of deposition was estimated by counting the number of spores per square centimetre on five standard microscope slides lightly greased with Plantex® (an adhesive substance used to trap insects and manufactured by ICI-Kynoch Agrochemicals). Each slide was marked with four different 1-cm^2 areas. One slide was placed at the centre of the turntable and four others on equally spaced radii in four different directions. Spore settling characteristics were tested by dispersing 1, 2, 4 and 8 mg of freshly harvested urediniospores of *U. viciae-fabae* downward into the tower as described above. The turntable speed was set to 14 revolutions per minute for all experiments.

The gravitation of *U. viciae-fabae* urediniospores in still air ranges from 0.78 to 1.58 cm/s (Clement, Porter and Beckett, 1998). If the cylinder height used in this study is divided by these velocities, the settling time for 120 cm ranges between 2 min 34 s and 1 min 16 s. Deposition for each spore quantity through gravitation was determined by counting spores per square centimetre on the microscope slides after a 3 min exposure period. The uniformity of the assay was determined in two independent runs.

In vivo deposition assay

Twenty seeds of the rust susceptible cultivar EL-142 were sown per 10-cm-diam. plastic pot filled with a 1:1 v/v steam sterilized soil/peat moss mixture. Pots were placed in a rust-free and air-conditioned glasshouse cubicle maintained at a 27/14 °C day/night cycle. Plants received natural daylight supplemented with illumination from cool-white fluorescent tubes, which supplied 120 $\mu mol/m^2.s^{-1}$ for 14 h each day. Seven days after planting and every three days thereafter, 50 ml of a 2-g/l

hydroponics nutrient solution (6.5-2.7-13, N-P-K plus microelements: 0.15-0.024-0.024-0.005-0.002-0.001, Fe-Mn-B-Zn-Cu-Mo) was added as a soil drench to each pot.

Before inoculation, urediniospores stored for 10 months at -72°C were heat shocked at 40°C for 6 min. Twelve-day-old plants (V6-V7 growth stage, Erskine *et al*., 1990) were inoculated in the settling tower as described previously by placing four pots (sub-replications) on a turntable, and discharging 8 mg of urediniospores from 1.2 m above the top of plants in three separate runs (replications). A 3 min settling time was allowed for each inoculation. To determine spore viability, two 9-cm petri dishes containing 1.2% water agar were placed on the turntable during each run.

Following inoculation, plants were removed from the settling tower and placed in a dew chamber (relative humidity > 96% at 18-22 °C under darkness for 24 h). Plants were removed from the dew chamber 24 h post-inoculation and then placed, after drying-off, on a glasshouse bench at an approximate night/day regime of 15/21°C. Plants received natural daylight supplemented with illumination as described previously. Pots were arranged in a randomized complete block design. Spore viability was determined by examining the number of germinating spores on the water agar that had been placed in the inoculation target area and incubated in the dark under conditions similar to inoculated plants. After the petri dishes were removed from the dew chamber, urediniospores were immediately killed and stained using lactophenol cotton blue (Ellison, Cullis and Kable, 1992). Five separate areas over each agar plate were scored for spore germination.

The number of uredinia on both surfaces of the first two bifoliate and two multifoliate leaves of two sample plants in each treatment was counted 10 days post-inoculation [d.p.i.]). Twelve d.p.i., sample leaves were photographed using a digital

camera (Nikon, Japan). Measurements of leaf area were then taken using image analysis software (Lamari, 2002). From these data, pustule densities were determined by dividing the total number of pustules on sample leaves by leaf area.

Data analyses

F-tests (tests of variance ratio) were performed to determine if there were significant differences between runs and among the different positions on the turntable for the amount of spores deposited. The relationship between amounts of spores dispersed and those deposited was established through correlation and regression analyses. MSTAT-C statistical software (Bricker, 1991) was used to perform the *F*-tests and regression analysis. Data on number of uredinia per square centimetre leaf area from the different replications and sub-replications were subjected to analysis of variance (ANOVA) to determine uniformity of rust infections.

RESULTS AND DISCUSSION

Since the ratio of the variances (*F* value) from the two runs for each of the spore concentrations was non-significant, the data were combined for the ANOVA. The combined ANOVA indicated that variation in spore deposition between locations was significant ($P \leq 0.05$) when 2 and 4 mg spore quantities were dispersed. With an 8 mg spore quantity, however, variation became non-significant ($P \geq 0.05$) (Table 4.1). The test also showed 1 mg to be non-significant, but the degree of precision was lower (coefficient of variation [CV] = 22%) when compared with the other spore quantities (Table 4.2). The deposition of spores increased linearly with quantity dispersed. The number of spores deposited was proportional to the mass dispersed ($Y = 116.25X$) where Y = number of spores deposited and X = mass of spores dispersed. The *F*-test

for this regression equation was highly significant ($P \leq 0.01$) and it explained about 98% of the variation in spore deposition (Table 4.3).

According to the standard deviations (SD) and CV uniformity of spore deposition improved with an increase in the quantity of spores dispersed (Table 4.2). The SD for the number of spores deposited per square centimetre was inversely proportional to the spore mass dispersed or mean deposition (Table 4.2). A similar inverse relationship occurred between the CV and the quantity of spores dispersed, with 22%, 17%, 7% and 5% being recorded for the 1, 2, 4 and 8 mg quantities (Table 4.2). These statistics are in agreement with those of Schein (1964) who had observed, in trials with urediniospores of *Uromyces phaseoli*, that the CV were 7-12% and 25% when mean depositions were greater than 100 and less than 100 spores per square centimetre, respectively.

Uniformity and consistency of infections are essential requirements of any method of inoculation used to compare host cultivars for receptivity or pathogen races for infectivity (Mortensen *et al*., 1979). These requirements are also necessary to make quantitative comparisons among treatments in epidemiological studies. This study demonstrated that the inoculation method used produced a uniform spore deposition on the target area, but that repeatability depended on the spore quantity.

In addition to the uniformity of deposition, viability of spores influences disease severity in experiments of this nature. In the *in vivo* assay, urediniospores retained a viability of $\geq 98\%$ after a 10-month storage period at -72°C. The analysis of variance showed that the number of lesions per square centimetre leaf area within and between runs was statistically similar (mean 140 ± 7 and CV 5%, Table 4.4). This indicates that the distribution of lesions was uniform (Fig. 4.2) and that this technique should be suitable for studies of lentil rust in the glasshouse.

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Table 4.1 Analysis of uniformity of deposition of urediniospores of *Uromyces viciae-fabae* during a 3 min settling period

Spores dispersed (mg)	Mean number of spores deposited/cm^2	*F*-value
1	81	1.830 ns[a]
2	244	5.997*
4	384	5.821*
8	972	1.401 ns

[a] ns = not statistically significant.
* Significant at the 5% level of probability.

Table 4.2 Number of urediniospores of *Uromyces viciae-fabae* per square centimeter on five Plantex®-coated slides placed horizontally on the turntable in a settling tower when four different quantities of spores were dispersed

Slide	Number of spores per dispersed quantity			
	1 mg	2 mg	4 mg	8 mg
1	76	264	434	979
2	89	283	368	963
3	98	262	360	992
4	79	167	355	924
5	66	-	405	1003
Mean	81	244	384	972
SD	18	42	28	52
CV (%)	22	17	7	5

Table 4.3 Analysis of regression of number of urediniospores of *Uromyces viciae-fabae* deposited per square centimeter on mass of the urediniospores dispersed into a settling tower

Source of variation	Degree of freedom	Mean squares	*F*-value	SE	Coefficient of determination (R^2)
Regression	1	445103	131**	58	0.978
Error	2	3397			
Total	3				

** Significant at the 1% level of probability.

Table 4.4 Analysis of uniformity of rust infection following exposure of lentil plants to urediniospores of *Uromyces viciae-fabae* in a settling tower

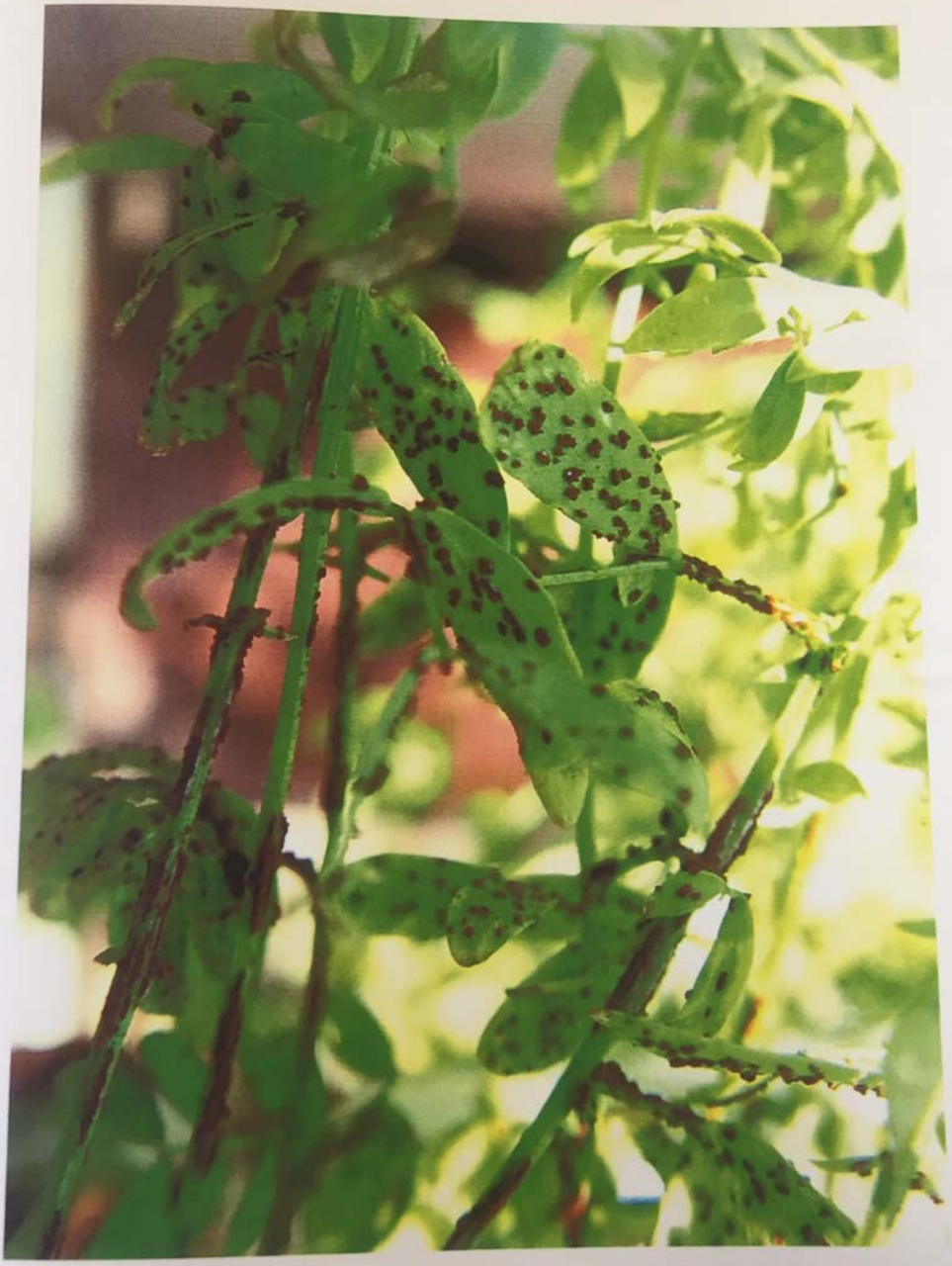
Source of variation	Degree of freedom	*F*-value	Significance*
Replication	2	0.3	ns
Sub-replication	3	2.0	ns
Error	6	-	-
Total	11		

* ns = non-significance at the 5% level of probability.

Figure 4.1 Image of the spore settling tower showing (A) side view of the tower. HS, hole and shutter; LD, lid; HA, handle, (B) the turntable with a motor. L, motor lever; and M, motor and (C) top view of the settling tower with lentil seedlings placed on the turntable.


HS
LD
HA
A
L
M
B
C

Figure 4.2 Lentil plants showing rust pustules on leaves and stems resulting from inoculation with 8 mg of urediniospores of *Uromyces viciae-fabae* for a 3 min settling period.



CHAPTER 5

EFFECT OF ENVIRONMENTAL FACTORS ON *IN VITRO* GERMINATION OF UREDINIOSPORES AND INFECTION OF LENTILS BY RUST

"Naturally, the fullest application of meteorological techniques will only be achieved where the mycologist can confer with the trained meteorologist."

P.M. Austin Bourke

ABSTRACT

For accurate lentil (*Lens culinaris*) rust phenotyping in controlled environments, conditions for infection should be optimized. Therefore, the effects of temperature on germination and germ tube growth of *Uromyces viciae-fabae*, as well as different dew periods, were quantified. In all experiments urediniospores of a single-pustule isolate were applied using a previously calibrated settling tower. After 3 h of incubation, a high percentage (≥ 80%) of spore germination was observed on 1.5% water agar at 10, 15, 20 and 25°C, with an optimum (99%) at 20°C. At this sampling time the length of germ tubes ranged from 66 µm (10°C) to 196 µm (20°C). Growth of germ tubes increased progressively from 10 to 20°C and then declined at 25°C. For minimum infection of lentil cultivar EL-142 at 20°C, a dew period of at least 3 h was required, whereas maximum infection occurred with a dew period of 24 h. Infection efficiency increased linearly as the duration of dew period increased from 0 to 24 h. Regression models that best described the quantitative relationship between the environmental variables and growth of the pathogen and development of rust were derived empirically. Such models are of significance in optimizing studies of the particular pathosystem as well as eventual lentil rust prediction models.

INTRODUCTION

Lentil rust, caused by *Uromyces viciae-fabae* (Pers.) J. Schröt. is responsible for considerable losses to lentils in several regions of the world (Beniwal *et al*., 1993). The use of rust-resistant lentil (*Lens culinaris* Medik.) cultivars is the most economically feasible and practical solution considered by resource-poor farmers, and screening for rust resistance continues to be a major objective of lentil breeding in countries where this disease is a problem (Erskine *et al*., 1994). Lentil lines are mostly evaluated for their resistance to rust under natural conditions (Erskine *et al*., 1994). However, weather conditions in the field are not always conducive to rust epidemics, and to sustain lentil-breeding programs, it is often desirable to evaluate genotypes in controlled environments.

Despite the need for more controlled infection studies, there is no standard protocol of inoculation techniques, temperature and dew period that facilitate consistent development of lentil rust. Few studies have dealt with the effect of environmental factors on infection and spore viability of *U. viciae-fabae*. Prasada and Verma (1948) reported how temperature affects infection and survival of spores stored at a wide range of temperatures and Kispatic (1950) related weather conditions to viability of spores. Kramm and Tay (1984) recommended a 72-h post-inoculation dew period to obtain maximum infection. However, none of these reports provided quantitative information concerning the effects of dew period and temperature, respectively, on specific components of lentil rust development and spore germination or germ tube growth. Quantitative information on the foregoing aspects generated from monocyclic experiments in controlled conditions are helpful to predict the effects of climatic factors on lentil rust epidemics in the field.

The objectives of this study were to quantify the effects of (i) temperature and incubation period on germination and germ tube growth, and (ii) post-inoculation dew period on infection of lentils by urediniospores of *U. viciae-fabae*.

MATERIALS AND METHODS

Effects of temperature and incubation periods on germination and germ tube growth

The effects of four temperatures, viz. 10, 15, 20, and 25°C on germination and germ tube growth of urediniospores of Akaki mono-pustule isolate 1 (AMPI1) of *U. viciae-fabae* incubated in the dark for 3, 6, 9 and 12 h were examined. The experiment was a 4 x 4 factorial in a randomized complete block design. The 1.5% water agar plates (9-cm-diam.) were uniformly inoculated with 8 mg dry freshly harvested urediniospores of AMPI1 by placing them in a previously calibrated spore-settling tower. This quantity of spores gave an even coverage of agar plates with urediniospores at about 973 spores/cm^2.

The first group of 16 plates (four plates/temperature) was removed from the growth chambers after 3 h and continued until the final group was removed after 12 h. Upon removal, urediniospores were immediately killed and stained using lactophenol cotton blue (Ellison *et al*., 1990). After removal of each group of agar plates, the percentage of germinated urediniospores was determined by microscopically counting four lots of spores (100 spores/lot) on each plate. A spore was scored germinated if it had a germ tube, which was as long as the shortest diameter of the spore (Zadoks, 1978), i.e. ca. 30 μm for *U. viciae-fabae*.

The length of germ tubes was measured at 100x magnification using a scale in a microscope eyepiece (reticule). The length of a germ tube in the ocular unit was then converted to micrometer according to the instructions of the stage micrometer manufacturer Graticules Ltd., Tonbridge, Kent, England. The length of 15 germ tubes selected at random across the agar surface was measured on each petri dish and these were averaged to represent a population of germlings/replication.

Data analyses

Analysis of variance (ANOVA) was performed to determine treatment differences. The presence of linear relationships between individual environmental factors and spore germination or germ tube growth was assessed using simple linear correlation analysis. The relationships between temperature and incubation period for spore germination and germ tube growth were described following multiple regression procedures. Linear, quadratic and cubic effects of temperature and incubation periods and their interactions on spore germination and germ tube growth were tested. Regression terms whose coefficients were not significantly different from zero ($P > 0.05$) were dropped from the original equation formulated with variables created from the two independent variables (Gomez and Gomez, 1984). The data were then refitted to the regression equation with the remaining regression terms. This test of significance of regression coefficients and refitting of the data continued until a model with the best parameter estimates was derived (an equation with significant R^2 and significant regression coefficients). The Number Crunching Statistical System software (NCSS 2000) was used to perform the analysis.

Effect of dew period on infection of lentil seedlings

Pathogen

Urediniospores of *U. viciae-fabae* isolate AMPI1 were maintained on cv. EL-142 until inoculation.

Host material

The effect of post-inoculation dew period on lentil cv. EL-142 was examined. Twenty seeds were planted in 36 10-cm-diam. plastic pots containing a sterilized soil : peat moss (1:1 v/v) mixture. The pots were placed in a rust-free and air-conditioned glasshouse compartment maintained at a 27/14°C day/night cycle. Plants received natural daylight supplemented with illumination from cool-white fluorescent tubes, which supplied 120 $\mu mol/m^2.s^{-1}$ for 14 h each day. Seven days after planting and every three days thereafter, 50 ml of 2-g/l hydroponic nutrient solution (6.5-2.7-13, N-P-K plus micronutrients) was added as a soil drench to each pot.

Inoculation procedure

Twelve-day-old seedlings (V6/V7 growth stage) (Erskine, Muehlbauer and Short, 1990) of cv. EL-142 were inoculated in a settling tower with freshly harvested urediniospores. Plants were inoculated by placing four pots (four replications), equidistantly from the center, on the turntable and forcibly discharging 8 mg of dry urediniospores into the tower using an air gun, and allowing a 3-min settling time. Deposition of viable spores was estimated by counting germinated spores per square centimeter on agar-coated microscope slides (Schein, 1962) placed in the settling tower during inoculation and incubated for 12 h in a dew chamber in conditions similar to the treatments.

Following inoculation, plants were removed from the settling tower and placed in a dew chamber in the dark at 18-22°C with a relative humidity of > 96%. Four pots of control seedlings did not receive dew treatment. Plants were removed from the dew chamber 3, 6, 9, 12, 18, 24, 36 and 48 h post-inoculation and then placed, after drying-off, on a glasshouse bench at an approximate day/night temperature regime of 22/18°C and supplemental lighting as described previously. There were four replications per dew period treatment and two plants (four leaves) per replication or pot arranged in a randomized complete block design. The experiment was conducted twice.

Numbers of pustules on sample leaves were counted daily starting five days post-inoculation until no further pustules appeared. Twelve days after inoculation, leaves of sample plants were photographed using a digital camera (Nikon, Japan), and leaf area was then measured using Assess® image analysis software (Lamari, 2002) for calculating infection efficiency. The infection efficiency was calculated using the formula by Schein (1962) as follows:

$$\text{IE} = \frac{Number\ of\ \ pustules / cm^2}{Number\ of\ viable\ spores / cm^2}$$

Data analyses

Differences among treatments were determined using ANOVA. Regression-model analyses and fitting were performed following a procedure similar to that used in the temperature experiment.

RESULTS

Temperature

Spore germination

Germination of urediniospores was significantly ($P \leq 0.05$) affected by temperature, duration of incubation period and by their interactions. Spore germination ranged from 80% for 10°C at a 3-h incubation period to 99% for 20°C at 12 h incubation period. As temperature increased from 10°C to 25°C, spore germination increased to a maximum of 99% at 20°C and then declined (Fig. 5.1A). Spore germination was not significantly affected by the duration of incubation at 20°C.

Spore germination occurred during a 3-h incubation period at all temperatures (Fig. 5.1A). Germination of urediniospores of *U. viciae-fabae* occurred 3 h after incubation in the range 10 to 25°C (Fig. 5.1B). At this incubation period, high levels of germination were attained ranging from 80% for 10ºC to 95% for 20°C. At all incubation temperatures, germination increased from 3 to 12 h. However, germination reached its peak within 6-12 h from 15 to 20°C (Fig. 5.1B). Germination increased from 10 to 20°C, and then declined as the temperature increased to 25°C (Fig. 5.1A). Germination percentage was significantly ($P \leq 0.05$) higher at 20°C than at the lower (10°C) and higher (25°C) temperatures (Fig. 5.1A). The relationship of germination (G, %) with length of incubation time (t, in hour) and temperature (T, in °C) was described by the following equation:

$$\%G = 21.44t + 13.56T - 0.89t^2 - 0.35T^2 - 2.21tT + 0.093t^2T + 0.056tT^2 - 0.0023t^2T^2 - 35.91$$

$$(R^2 = 0.95, P \leq 0.01)$$

Germ tube growth

Germ tube growth was observed at all temperatures and incubation times. The maximum mean tube length of 422 µm was reached at 20°C (Fig. 5.2A). There were significant differences ($P \leq 0.05$) between incubation temperatures for germ tube growth (Fig. 5.2B). After 3 h incubation, the length of germ tubes ranged between 66 µm at 10°C and 196 µm at 20°C (Fig. 5.2A). Germ tube length increased progressively from 10 to 20°C and then declined at 25°C (Fig. 5.2B). Germ tube length reached its maximum at 12 h of incubation time at all incubation temperatures (Fig. 5.2A). At this sampling stage, average germ tube lengths were 197, 232, 422 and 214 µm, respectively, for 10, 15, 20 and 25°C (Fig. 5.2A).

Among the models tested, a quadratic equation adequately described germ tube growth (G, in µm) as a function of temperature (T, in °C) and incubation period (t, in hour). The model is:

$$\mathbf{G = 12.28t + 72.97T + 0.0191t^2 - 1.98T^2 - 494.90 \quad (R^2 = 0.72, P \leq 0.01)}$$

Dew period and infection

Duration of dew period had a significant ($P \leq 0.05$) (Fig. 5.3A) influence on disease development. At 20°C, 3 h was the minimum dew period that permitted infection in the glasshouse (Fig. 5.4B). Infection increased with increasing dew period (Figs. 5.4B, 5.4C, 5.4D, 5.4E) and reached a maximum at 24 h of leaf wetness (Fig. 5.3A). Infection efficiency was significantly ($P \leq 0.05$) affected by the post-inoculation dew period (Fig. 5.3B). Infection did not occur without dew (0 h dew period) (Fig. 5.4A). The final pustule number per leaf increased from 0 pustules/leaf at 0 h to 157 pustules/leaf at 24 h and then declined to 118 and 111 pustules/leaf at 36 and 48 h of dew periods, respectively (Fig. 5.3A).

A simple linear correlation coefficient between the two variables (dew period and number of pustules/leaf) was non-significant ($r = 0.51$, $P \geq 0.05$). Hence, nonlinear functional forms were examined to determine the type of their relationship. The response of this parameter to dew period was best described by a cubic equation ($R^2 = 0.99$, $P \leq 0.05$) (Fig. 5.3A). Infection efficiency increased as dew period increased from 3 h (0.004) to 12 h (0.09). Linear regression was used to quantify the relationship between dew period and infection efficiency, and dew period explained 91% of the variation in infection efficiency ($P \leq 0.01$) (Fig. 5.3B). Infection efficiency increased by 0.009 for each 1 h increase in the duration of leaf wetness.

DISCUSSION

In the present study, urediniospores of *U. viciae-fabae* germinated over a temperature range of 10 to 25°C, with a maximum germination at 20°C. It was clear, however, that the process is slowed down at 10°C and 25°C. Several reports (Ellison *et al*., 1990; Ellison, Cullis and Kable, 1992; Menniti, 1993; Shi, 1996) indicate that temperature has an influence on germination and growth of urediniospores of many rust fungi. With *U. viciae-fabae* on faba bean (*Vicia faba*) a comparable temperature range and optimum were reported (Joseph and Hering, 1997).

Although the length of germ tubes in individual spores reached as high as 650 µm (data not shown), average germ tube length is considered a more realistic growth estimate of a population of urediniospores in a field epidemic. Germ tube growth was similar to spore germination in its response to temperature. The slowing of germ tube growth and spore germination below and above 20°C indicates that the effect of temperature on basic biochemical reactions occurring within rust spores during the two developmental stages of *U. viciae-fabae* appears essentially similar. It is known

that temperature affects specific enzymes involved in spore germination (Gottlieb, 1978).

The data from this study demonstrated that the duration of dew period is an important factor for rust development on lentils. There was a large increase in disease development in plants incubated for 24 h, suggesting this to be the optimal period for lentil infection by the rust. Similarly, studies of other diseases (Makowski, 1993; Monroe, Santini and Latin, 1997; Groove, 2002; Erincik *et al*., 2003; Holtslag *et al*., 2003;) also revealed a pattern of increased disease with increasing dew period. In this study, the number of *U. viciae-fabae* pustules per leaf increased with an increasing dew period up to 24 h, and this result is consistent with that reported for infection of pea by *U. viciae-fabae* (Chauhan and Singh, 1994). With ascochyta blight of lentil (*Ascochyta fabae* f. sp. *lentis*), Pedersen and Morall (1994) and anthracnose of lentil (*Colletotricum truncatum*), Chongo and Bernier (2000) reported a dew period of 24 h and temperature of 20°C as conditions required for optimum disease development. Webb and Nutter (1997) reported that infection efficiency of *U. striatus* on alfalfa increases with increasing duration of leaf wetness up to 24 h after incubation, further substantiating present results. Therefore, it is not only the dew *per se* that is important for rust development, but its duration will also play a significant role in the amount of infections at a particular temperature.

In the present study no infection was obtained in plants not exposed to surface moisture, whereas slight infection occurred after a 3 h dew period. A similar effect of leaf wetness duration has been reported for *U. viciae-fabae* in faba bean. Joseph and Hering (1997) showed that infection of broad beans by *U. viciae-fabae* was not possible with < 4 h of continuous leaf surface moisture. However, some fungal pathogens are capable of infecting their hosts in the absence of leaf wetness. Trapero-

Casas and Kaiser (1992) showed that infection of ascochyta blight (*Ascochyta rabiei*) in chickpeas (*Cicer arietinum*) occurs without a wetting period after inoculation, a scenario not found with *U. viciae-fabae* in lentil.

The models fitted to the data may be of importance in characterizing the germination process in fields where conditions similar to those in the present study prevail. Obviously, the regression equations developed here apply to controlled environmental conditions, but they lay the foundation for the development of disease forecasting models in the field. However, for the models to be useful, they need to be validated. This work is neither definitive nor comprehensive, but could be considered as a step forward in a more detailed analysis of the lentil-rust pathosystem.

Urediniospores of *U. viciae-fabae* can germinate and infect lentils within 3 h of incubation period provided the post-inoculation conditions, which prevailed in the present study, are fulfilled. This suggests that the environmental conditions for the growth of *U. viciae-fabae* and rust disease development in lentil are essentially similar, underlining its great potential in causing field epidemics.

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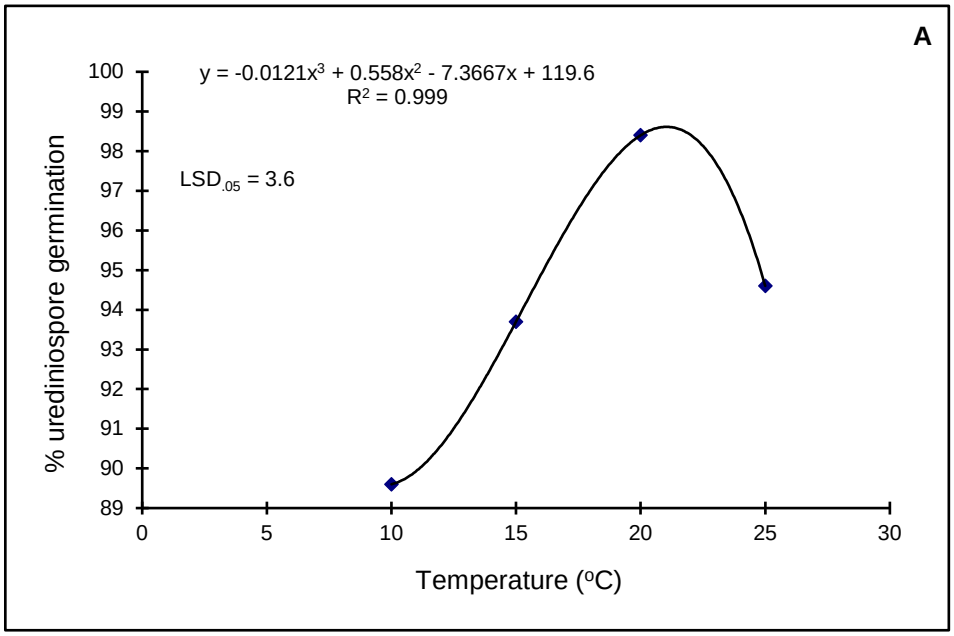
Figure 5.1 *In vitro* germination of urediniospores of *Uromyces viciae-fabae* as affected by (A) incubation temperature and (B) incubation time.


A
y = -0.0121x3 + 0.558x2 - 7.3667x + 119.6
R2 = 0.999
LSD.05 = 3.6
% urediniospore germination
100
99
98
97
96
95
94
93
92
91
90
89
0
5
10
15
20
25
30
Temperature (ºC)

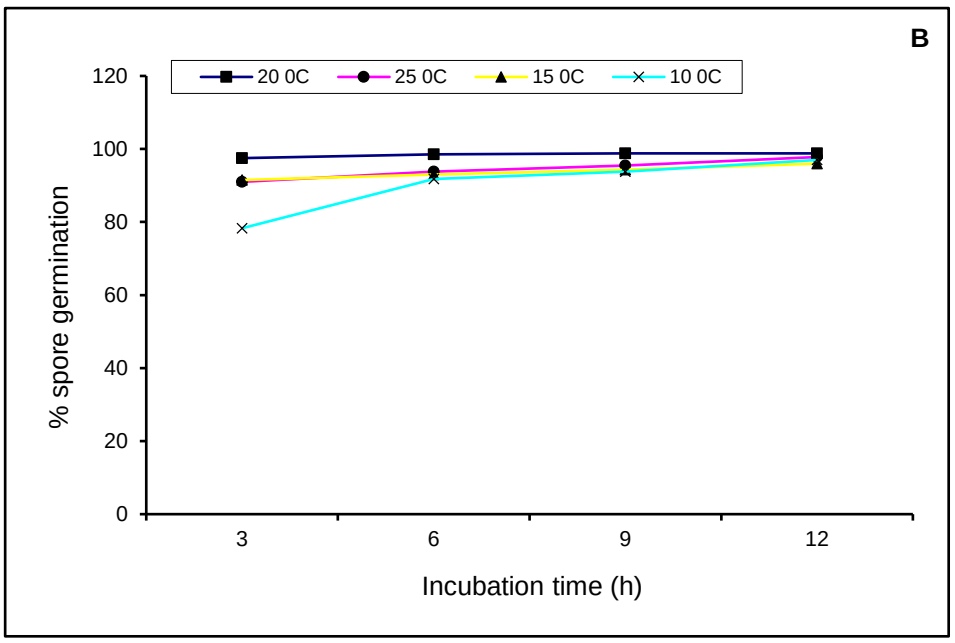
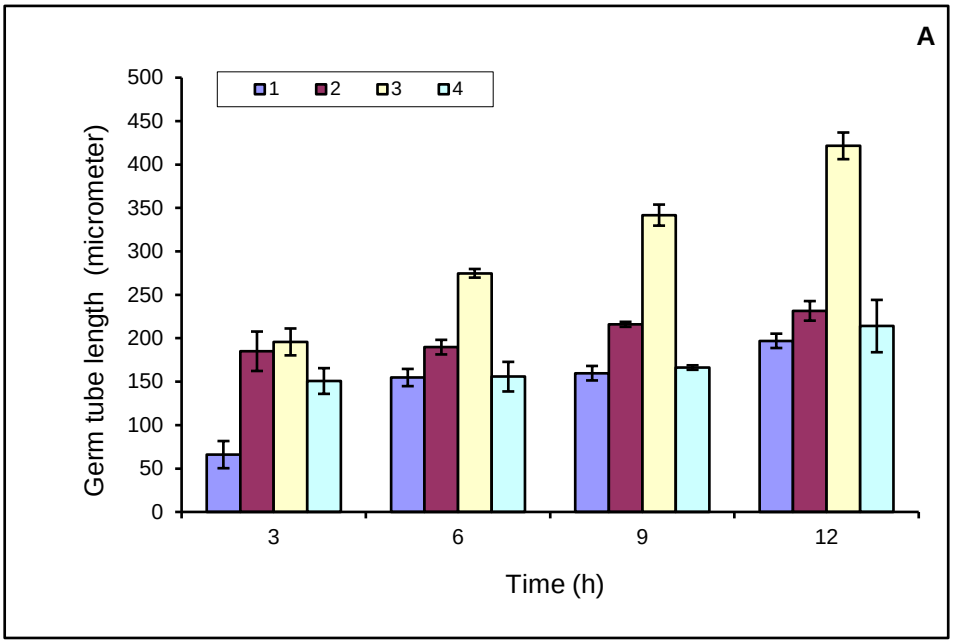

B
20 0C
25 0C
15 0C
10 0C
% spore germination
120
100
80
60
40
20
0
3
6
9
12
Incubation time (h)

Figure 5.2 Length of germ tube of urediniospores of *Uromyces viciae-fabae* as influenced by (A) incubation temperature and time; each point is an average length of 60 germ tubes; error bars indicate standard deviations; 1 = 10°C, 2 = 15°C, 3 = 20°C, and 4 = 25°C, and (B) temperature.


A
1
2
3
4
Germ tube length (micrometer)
500
450
400
350
300
250
200
150
100
50
0
3
6
9
12
Time (h)

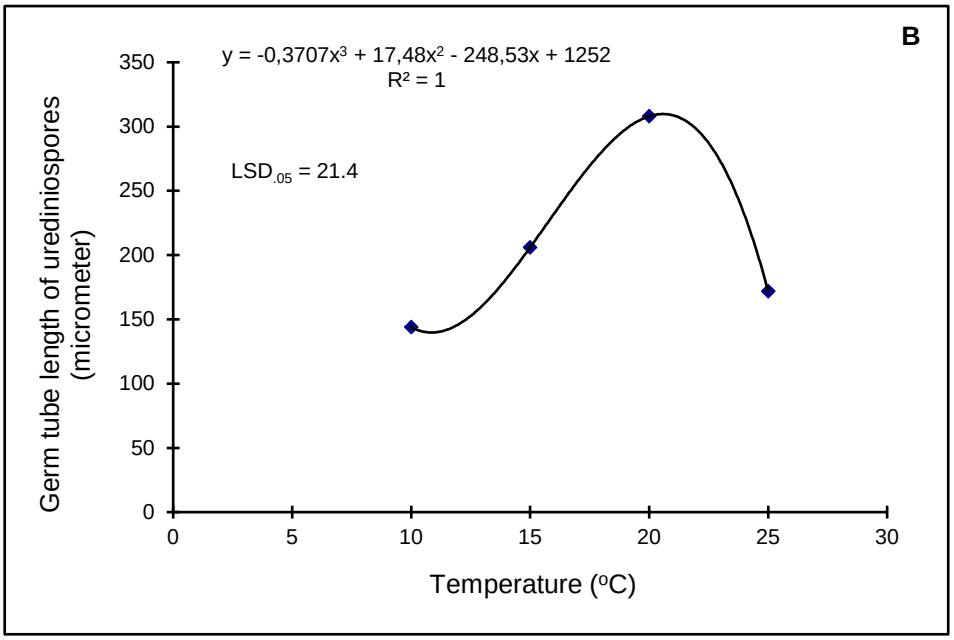
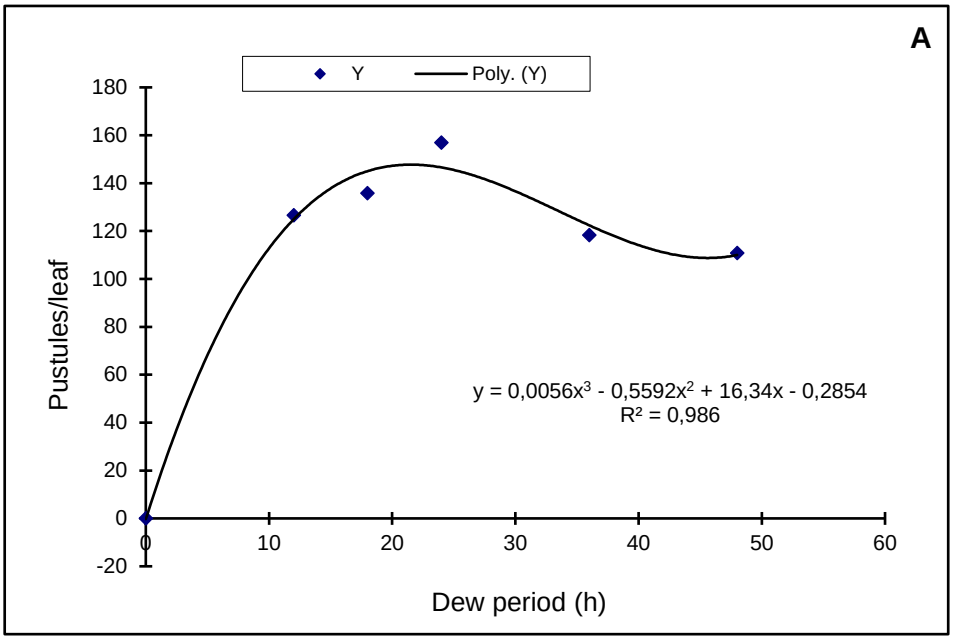

B
y = -0,3707x³ + 17,48x² - 248,53x + 1252
R² = 1
LSD.05 = 21.4
Germ tube length of urediniospores (micrometer)
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
Temperature (°C)

Figure 5.3 Relationship between dew period and rust parameters in a monocyclic infection study: (A) final pustule number/leaf of *Uromyces viciae-fabae* on lentil cultivar EL-142 as a polynomial function of dew period, and (B) linear regression of infection efficiency of *Uromyces viciae-fabae* on dew period (h).


A
Y
Poly. (Y)
Pustules/leaf
180
160
140
120
100
80
60
40
20
0
-20
0
10
20
30
40
50
60
Dew period (h)
y = 0,0056x³ - 0,5592x² + 16,34x - 0,2854
R² = 0,986

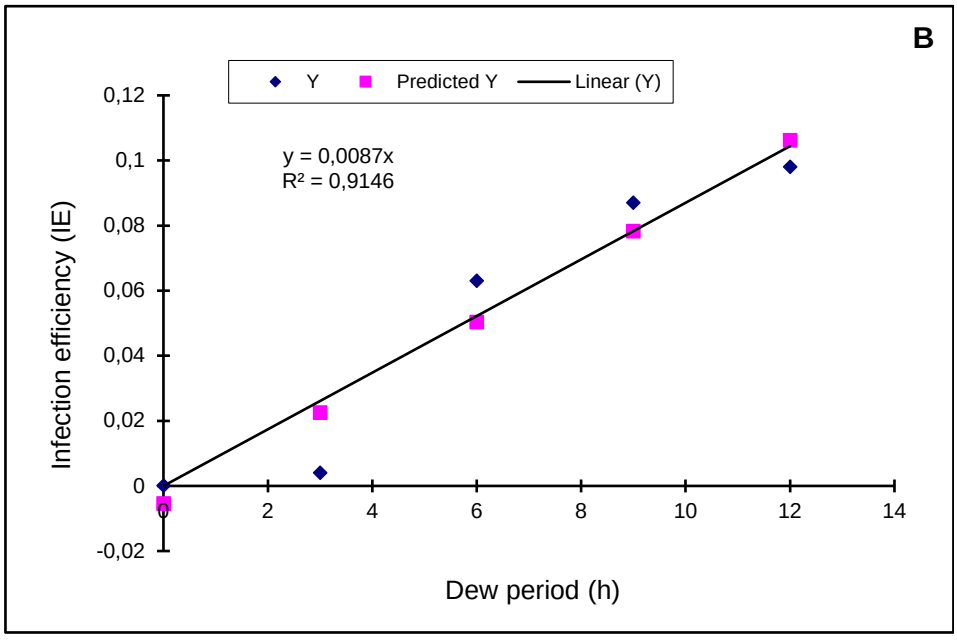

B
Y
Predicted Y
Linear (Y)
Infection efficiency (IE)
0,12
0,1
0,08
0,06
0,04
0,02
0
-0,02
0
2
4
6
8
10
12
14
Dew period (h)
y = 0,0087x
R² = 0,9146

Figure 5.4 Effect of dew period on infection of lentil plants by *Uromyces viciae-fabae* at 20ºC (A) 0 h (B) 3 h (C) 6 h (D) 9 h and (E) 12 h.


A
25/8/03
0 hr
B
25/8/03
3hr
C
25/8/03
6 hr
D
25/8/03
9 hr
E
25/8/03
12 hr

CHAPTER 6

COMPONENTS OF RESISTANCE TO *UROMYCES VICIAE-FABAE* IN LENTIL

"The continued success of the "green revolution" will hinge largely on our ability to maintain adequate resistance to any array of plant pathogens which actually hold the key to continued crop production."

R.R. Nelson

ABSTRACT

Four components of resistance to *Uromyces viciae-fabae*, namely, latent period, infection efficiency, pustule size and spore production capacity were evaluated on lentil (*Lens culinaris*) in glasshouse experiments. Four cultivars, Gudo, R-186, FLIP-87-66L and FLIP-89-60L, with different levels of resistance, and the susceptible check EL-142, were included in this study. The cultivars were also compared for area under the disease progress curve (AUDPC), area under the pustule density curve (APDC), apparent infection rate (r_G), disease severity, pustule density, latent period and pustule size under field conditions. Gudo and R-186 had significantly smaller and fewer pustules, lower spore yield and longer latent period than EL-142. FLIP-87-66L was intermediate for infection efficiency and pustule size. In addition, significant differences were found between cultivars for AUDPC, APDC, disease severity and r_G. Estimates of AUDPC, APDC, disease severity and r_G were reduced in Gudo, R-186 and FLIP-87-66L compared with the susceptible check EL-142. FLIP-89-60L also showed low AUDPC, APDC and disease severity. Some of the components obtained in the field were significantly correlated with each other ($r = 0.92$-0.99, $P \leq 0.05$) and those measured in the glasshouse. Most of the components studied in the glasshouse were significantly ($P \leq 0.05$) correlated with AUDPC and disease severity. Data indicated the existence of incomplete (partial) resistance in the test cultivars, and the possibility of using infection efficiency, latent period, spore production capacity and pustule size as selection criteria in the evaluation of partial resistance to rust in lentil. Since there was an interdependence of the components, selection based on more than one component helps obtain lines with higher levels of partial resistance. The AUDPC, disease severity and r_G could also be used for selecting lines with partial resistance in the field.

INTRODUCTION

In Ethiopia where lentil (*Lens culinaris* Medik) is grown, rust [caused by *Uromyces viciae-fabae* (Pers.) J. Schröet] is considered as one of the major diseases causing yield losses of up to 100% (Negussie, Bejiga and Million, 1998). The majority of farmers' or traditional varieties (landraces) are susceptible or have little resistance to rust. Presently, control of lentil rust in Ethiopia includes the use of resistant varieties, developed at the Debre Zeit Agricultural Research Center (DZARC), Ethiopia, in collaboration with the International Centre for Agricutural Research in the Dry Areas (ICARDA).

Most studies on the genetics of rust resistance in lentil have revealed that resistance is monogenic and dominant (Sinha and Yadav, 1989; Singh and Singh, 1992). More recently, Chahota, Gupta and Sharma (2002) reported that resistance to rust is controlled by two duplicate, non-allelic and non-linked dominant genes. Although a break down of rust resistance in lentil has not been reported, the likelihood of such an event in varieties with monogenic resistance cannot be ruled out. Continuous cultivation of varieties with race-specific resistance in large areas increases selection pressure on the pathogen that may eventually lead to the formation of new races capable of infecting the previously resistant varieties.

Studies have shown that *U. viciae-fabae* is variable. For example, Singh *et al.* (1995) have reported five races, and Conner and Bernier (1982b) detected 11 races of the pathogen. In addition, species of *Vicia*, *Lathyrus* and *Pisum* could be important sources of inoculum and perhaps pathogenic variants (Conner and Bernier, 1982b). This suggests that specific resistance is temporary and efforts to improve the crop should focus on durable resistance.

There are several strategies of developing varieties with durable resistance. These include multilines (Marshall, 1977), partial resistance/slow rusting (Wilcoxson, Skovmand and Atif, 1975) and gene pyramiding (Green, 1975; Pederson and Leath, 1988). Partial resistance has been observed in food legumes for *Uromyces* rusts (Habtu and Zadoks, 1995; Conner and Bernier, 1982a) and for anthracnose (Chongo and Bernier, 1999). The phenomenon of partial resistance to rust in lentil has not been explicitly reported in the literature and little is known about its durability and inheritance. However, numerous lentil lines with varying levels of resistance have been identified in different countries (Singh and Sandhu, 1988; Bejiga *et al.*, 1995).

Partial resistance is a form of incomplete resistance that reduces spore production despite host plants being susceptible to infection (Parlevliet, 1979). However, partial resistance can also be expressed in any one phase of the infection cycle of a pathogen and built as components of resistance in a given cultivar (Zadoks and Schein, 1979). Development of lentil varieties with such a type of resistance, therefore, requires an understanding of the components of resistance to rust so that effective and reliable selection can be established.

In an attempt to implement this approach in other crops, several components of resistance have been evaluated. These include latent period, infection efficiency, spore production capacity, infectious period, pustule size, and pustule density (Ohm and Shaner, 1976; Parlevliet, 1979; Conner and Bernier, 1982a; Habtu and Zadoks, 1995). Values of disease severity, areas under the disease progress curve (AUDPC) and apparent infection rate (*r*) have been used to measure the expression of components of PR and compare different genotypes with varying levels of resistance in the field (Rashid and Bernier, 1986; Abdhikari *et al.*, 1999). Since lentil is a field crop, at least a field study approach needs to be followed (Aust and Kranz, 1988).

However, weather conditions are not always conducive for a rust epidemic in the field, and, hence, characterization of the components should also be done in controlled environments.

The objectives of this study were to: (1) detect the existence of partial resistance to rust in lentil; (2) characterize the components of resistance under field and glasshouse conditions; (3) relate the components among themselves within and between the field and glasshouse experiments; and (4) relate the components to disease severity and parameters of disease progress such as AUDPC and *r* values.

MATERIALS AND METHODS

Glasshouse experiment

The glasshouse study was carried out at the University of the Free State, South Africa. Ten seeds of each of the cultivars (cvs.) FLIP-89-60L, FLIP-87-66L, R-186 and FLIP-84-78L (Gudo) were sown in 10-cm-diam plastic pots filled with a 1:1 v/v steam sterilized soil/peatmoss mixture. EL-142 was included as a susceptible check. The pots were placed in a rust-free and air-conditioned glasshouse cubicle maintained at a 27/14 °C day/night cycle. Plants received natural daylight supplemented with illumination from cool-white fluorescent tubes, which supplied 120 $\mu mol/m^2.s^{-1}$ for 14 h each day. Seven days after planting and every three days thereafter, 50 ml of a 2-g/l hydroponic nutrient solution (6.5-2.7-13, N-P-K plus microelements: 0.15-0.024-0.024-0.005-0.002-0.001, Fe-Mn-B-Zn-Cu-Mo) was added as a soil drench to each pot.

Inoculum multiplication

Urediniospores of *U. viciae-fabae* were obtained from diseased lentils at Akaki, Ethiopia. A pure culture of urediniospores of Akaki mono-pustule isolate 1 (AMPI1) of *U. viciae-fabae* was increased on the cv. EL-142.

Inoculation and incubation

Twelve-day-old plants (V6-V7 growth stage) (Erskine, Muehlbauer and Short, 1990) were inoculated in a previously calibrated settling tower by placing four pots on a turntable, and forcibly discharging 8 mg of dry, freshly harvested urediniospores from 1.2 m above top of the plants into the tower. A 3-min settling time was allowed between successive inoculations. To determine inoculum density, four 9-cm petri dishes containing 1.2% water agar were placed on the turntable during inoculation.

Following inoculation, plants were removed from the settling tower and placed in a dew chamber (relative humidity > 96% at 18-22 °C under darkness for 24 h). Uninoculated plants of each cultivar were included to check if rust contamination had occurred before inoculation. Plants were removed from the dew chamber 24 h post-inoculation and then placed, after drying-off, on a glasshouse bench at an approximate night/day regime of 17/23 °C. Plants received natural daylight supplemented with illumination as described previously. Pots were arranged in a randomized complete block design with five replicates. The experiment was conducted twice.

Inoculum density (number of viable urediniospores deposited/cm^2) (Petersen, 1959) was determined by examining the number of germinating spores on the water agar that had been placed in the inoculation target area and incubated in the dark under conditions similar to inoculated plants. After the petri dishes were removed

from the dew chamber, urediniospores were immediately killed and stained using lactophenol cotton blue (Ellison, Cullis and Kable, 1992). Sixteen 1 cm^2 agar discs cut from the agar plate were scored for spore germination.

Rust measurements

The latent period was determined as the time from inoculation to when 50% of the terminal number of pustules were visible on the leaf surface (Parlevliet, 1975). The latent period was measured on the first two bifoliate and two multifoliate leaves of two sample plants in each treatment by counting the number of visible pustules daily from day six after inoculation until no more pustules appeared (11 days post-inoculation [d.p.i.]). From these data, latent period values were estimated by solving linear regression equations calculated from probit-transformed percent visible pustules (Shaner, 1980):

$$\text{Probit } Y = bX + a$$

In which Y = proportion of pustules visible, X = number of days after inoculation, b = slope of the line, and a = the Y-intercept. Probit transformations for each day of observation were made using the statistical tables published by Fisher and Yates (1974). Twelve d.p.i., sample leaves were photographed using a digital camera (Nikon, Japan). Measurements of leaf area and final lesion number were then taken by using image analysis software developed by Lamari (2002). The accuracy of pustule numbers counted with this program was verified by comparing them with those counted manually. After photographs had been taken, the diameters of 40 (20 on upper and 20 on lower leaflet surfaces) randomly selected pustules per treatment were measured manually with a comparator (Edmund Scientific Co., U.S.A.). The pustule

diameter was estimated as average of length plus width divided by 2. Pustules were assumed to be circular and areas were calculated accordingly. Thirteen d.p.i., pustule numbers on randomly-selected multifoliate and bifoliate leaves, one each per treatment, were counted. Leaves were then removed and carefully transferred into 20-ml vials containing 0.8 ml light mineral oil. Urediniospores were washed off the pustules by gently agitating the vials containing the leaf samples for 1 min and spores were counted using a Neubauer improved bright-line haemocytometer (Marienfeld, Germany) (depth = 0.100 mm and area = 0.0025 mm^2). Number of spores on each leaf was estimated by averaging six spore counts. The number of spores per pustule at 13 d.p.i. was then calculated by dividing the total number of spores on sample leaves by the total number of pustules on the leaves.

Infection efficiency was computed with the following formula (Schein, 1964):

$$IE = \frac{number\,of\ pustules/cm^2}{number\,of\ viable\ urediniospores/cm^2}$$

Infection type was scored 11 d.p.i. using the scale of Roelfs, Singh and Saari (1992).

Field experiment

Plant material and experimental design

The field study was conducted at Akaki, Ethiopia in 2001. Test cultivars were the same as those used in the glasshouse experiment. Each genotype was sown in a 9-m^2 plot containing ten 3-m rows with 60 seeds/row and 30 cm between rows. The experimental design was a randomized complete block with four replications. Each plot was surrounded by five guard rows of wheat to minimize interplot interference

(Aust and Kranz, 1988). Moreover, plots were made square shaped (3 m × 3 m) to lessen inoculum exchange between plots (Paysour and Fry, 1983). An uninoculated plot of the whole set of the test cultivars was included at the end of each replication to check if there was any contamination with rust that had occurred before inoculation. The plots and blocks were separated by a 2-m and 2.5-m space, respectively.

Inoculation

Plants were inoculated at the V9-V10 growth stage (Erskine *et al.*, 1990), four weeks after planting, with urediniospores of the Akaki rust isolate. The concentration of the spore suspension was measured using a haemocytometer and adjusted with distilled water to 3 x 10^4 urediniospores ml^{-1} (Kramm and Tay, 1984), and each plot was inoculated with ca. 1 litre of the suspension using a knap-sack sprayer. Tween 20® (polyoxyethylene sorbitan monolaurate) was added as a wetting agent at a rate of one drop per 1000 ml of the suspension (Chongo and Bernier, 1999). Control plots were inoculated with water and Tween 20® only. Following inoculation wooden frames were placed in plots and covered with clear polyethylene sheets overnight. The inoculation was made after a shower of rain when the soil in each plot was wet enough to create a high relative humidity during incubation (Chongo and Bernier, 1999).

Assessment of resistance components

Disease was assessed weekly for each cultivar on the same 12 randomly selected plants per plot.

Disease severity

Disease severity was recorded using a 9-point disease rating scale (Khare, Bayaa and Beniwal, 1993). Disease severity was recorded eight times at weekly intervals starting one week after inoculation. Percent rust severity (% RS) per plot was then calculated using the following formula by Chongo, Bernier and Buchwaldt (1999):

$$\%RS = \frac{\sum[no.of\ plants/scale \times scalevalue]}{highest\ scale \times total\ no.of\ plants} \times 100$$

Changes in disease severity with time were plotted. The sigmoid curves of disease progress over time were linearized using the Gompertz transformation of the severity values (Berger, 1981). The resulting lines were straight and the apparent infection rates (r_G) were determined from the slope values of the simple linear regressions of the transformed disease proportions that occurred over time. The Gompertz model was selected over the logistic based on goodness of fit for the 20 disease progress curves constructed from this experiment. In general, the standard error of the estimate (a measure of the variability of *y* values about the regression line) and coefficient of determination (R^2) were respectively smaller and higher for the Gompertz model.

The AUDPC values were calculated from disease severity data obtained from the field using the formula of Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n}\left[(Y_{i+n1}+Y_i)/2\right]\left[T_{i+1}-T_i\right]$$

Where Y_i is rust severity (per unit) at the *i*th observation, T_i is time in days at the *i*th observation, and *n* is the total number of observations.

Latent period

Development of rust signs was inspected daily starting 3 d.p.i. Numbers of pustules per cultivar were counted daily once they were observed until no further increase in pustule number occurred for two consecutive days. Counts were made on leaflets of the 12 sample plants per plot.

Pustule density

For the purpose of determining pustule density, numbers of pustules were counted per plant on each cultivar eight times at weekly intervals starting one week after inoculation. Pustule density was then calculated by dividing the number of pustules by the total leaf area per corresponding sample plant. The changes in the number of pustules over time were plotted to see variation among cultivars.

By analogy to the area under the disease progress curve, the area under the pustule density progress curve (APDC) was calculated from pustule density data as follows:

$$\text{APDC} = \sum_{n=1}^{n} \left[\left(P_{i+1} + P \right) / 2 \right] \left[T_{i+1} - T_i \right]$$

Where P_i = pustule count (per unit) at the *i*th sampling date and T_i = time (in days) at the *i*th observation and *n* = total number of observations.

Pustule size

Pustule size was assessed on each of the 12 sample plants using a 4-point scale (Conner and Bernier, 1982b), where 0 = no sign of infection; 1 = uredinia minute barely sporulating; 2 = uredinia of about 0.5 mm diameter; 3 = uredinia of about 1 mm; and 4 = uredinia > 1 mm.

Crop assessment

The leaf areas of the sample plants were estimated weekly by means of calibrated grids printed on transparencies (Zadoks and Schein, 1979).

Data analyses

Analyses of variance were used to determine differences among lines for each component and for disease severity, AUDPC and APDC values. Mean comparison was done using the least significant difference (LSD, $P \leq 0.05$). Variance homogeneity tests were performed for each variable before subjecting data to ANOVA. Correlation analyses were made to compare the components among themselves and also to relate them to disease severity and AUDPC values. All statistical analyses were performed using MSTAT-C (Bricker, 1991).

RESULTS

Glasshouse experiment

Latent period

Latent period varied from 8.3 days on cv. EL-142 to 9.0 days on cv. Gudo. The latent period values for cv. R-186 and cv. Gudo were significantly ($P \leq 0.05$) longer than for the remaining cultivars (Table 6.1). Differences between cvs. R-186 and Gudo were non-significant. Similarly the differences among the other three cultivars were not significant.

Pustule size

Significant differences ($P \leq 0.05$) in pustule area (size) were found between cultivars (Table 6.1). Pustules were largest (0.56 mm^2) on cv. EL-142, intermediate (0.26

mm^2) on cv. FLIP-87-66L and smallest (0.096-0.098 mm^2) on cvs. Gudo and R-186. Cultivars ranked similarly for infection type where pustules were well formed without macroscopically visible necrosis or chlorosis (infection type = 4) on EL-142 and FLIP-89-60L to small pustules with macroscopically visible necrotic halo (infection type = 2) on cvs. Gudo and R-186. Leaf necrosis was frequently associated with pustules on the latter two cultivars.

Infection efficiency and spore production

Average infection efficiencies and spores produced per pustule are shown in Table 6.1. Infection efficiency of the pathogen was significantly ($P \leq 0.05$) higher in cvs. EL-142 and FLIP-89-60L than in the other cultivars. The number of spores produced per pustule on cv. EL-142 was also significantly ($P \leq 0.05$) higher than those on cvs. FLIP-87-66L, R-186 and Gudo.

Coefficients of correlation

Linear correlation coefficients between the components of resistance are shown in Table 6.2. A positive and strong correlation was found between number of spores per pustule and pustule area ($r = 0.94$, $P \leq 0.01$). Latent period was significantly ($P \leq 0.05$) and negatively correlated with IE and SP (Table 6.2).

Field experiment

Latent period

Significant ($P \leq 0.05$) differences in the latent period were observed among the test cultivars, and it was shortest for the susceptible cv. EL-142 (Table 6.3). Latent periods for FLIP-89-60L and FLIP-87-66L were approximately 1.85 times as long as

that for EL-142. On cv. EL-142, 50% of the visible pustules appeared eight days after inoculation, whereas, the same amount of pustules appeared 15 days after inoculation on cvs. FLIP89-60L and FLIP87-66L.

Pustule density

Significant differences between cultivars were found (Table 6.3). Pustule density varied from 0.1 pustules cm^{-2} on Gudo to 10 pustules cm^{-2} on EL-142. Gudo and R-186 had significantly fewer pustules per square centimeter of leaf area than FLIP-87-66L, FLIP-89-60L and EL-142, whereas FLIP-87-66L and FLIP-89-60L had significantly fewer pustules than cv. EL-142.

Pustule size

Difference between EL-142 and FLIP-89-60L for pustule size was non-significant. EL-142 and FLIP-89-60L had significantly ($P \leq 0.05$) larger pustules than FLIP-87-66L, R-186 and Gudo (Table 6.3). Pustule sizes were largest (ca. 1.0 mm in diameter) on EL-142 and FLIP-89-60L, intermediate (0.5-1.0 mm in diameter) on FLIP-87-66L, and smallest (≤ 0.5 mm in diameter) on R-186 and Gudo (Table 6.3). Pustules on the latter two cultivars were only four-tenths to six-tenths the size of those on EL-142 and FLIP-89-60L.

Rust severity, apparent infection rates (r_G) and AUDPC

Estimates of rust severity, r_G and AUDPC significantly ($P \leq 0.05$) varied with the lentil cultivars (Table 6.3). The r_G values were highest in EL-142 and FLIP-89-60L, intermediate in FLIP-87-66L and R-186, and lowest in Gudo. The final rust severity values ranged from 7.3% on Gudo to 65.4% on EL-142. EL-142 had the highest RS

value and the second highest RS value was for FLIP-89-60L followed by RS values for FLIP-87-66L, R-186 and Gudo in that order. Cultivars ranked similarly for AUDPC. Progress of rust was slower on Gudo, R-186 and FLIP-87-66L than on EL-142 and FLIP-89-60L (Fig. 6.1). The estimated curves were essentially straight (Fig. 6.2). The apparent infection rates (r_G) were determined from the slope values that occurred over time of the simple linear regression of the transformed disease proportions.

Cultivars Gudo, R-186 and FLIP-87-66L had slow disease progress and consequently had significantly ($P \leq 0.05$) lower AUDPC values than the susceptible check (Table 6.3). Although not significant, the r_G value was reduced in cv. FLIP-89-60L in comparison with EL-142. That small reduction in r_G ultimately contributed to a significantly ($P \leq 0.05$) lower AUDPC value in cv. FLIP-89-60L when compared to that in EL-142.

Coefficients of correlation

Latent period and pustule density were significantly and negatively correlated (r = -0.99). Latent period was also significantly and negatively correlated with pustule size, APDC, rust severity and AUDPC (Table 6.4). Pustule size was significantly and positively correlated with rust severity, AUDPC and r_G. Final rust severity was well correlated with APDC, AUDPC and r_G. APDC and AUDPC were also significantly correlated (r = 0.99). Apparent infection rate r_G, however, was not significantly correlated with latent period, pustule density and APDC. Thus, pustule size was the only component of resistance significantly correlated with r_G (r = 0.97) (Table 6.4).

Coefficients of correlation between glasshouse and field data

Coefficients of correlation between components of resistance measured in the glasshouse and field are shown in Table 6.5. Latent period measured in the glasshouse was significantly ($P \leq 0.05$) correlated with the latent period ($r = 0.86$), pustule size ($r = -0.97$), rust severity ($r = -0.95$) and AUDPC ($r = -0.84$) measured in the field. Spore production in the glasshouse was significantly correlated with all components in the field, ADPC, rust severity, and AUDPC, except r_G. None of the other components measured in the glasshouse was significantly correlated with r_G, as well. Infection efficiency was significantly and positively correlated with pustule size and rust severity in the field. All the components measured in the glasshouse were significantly correlated with rust severity in the field.

DISCUSSION

The cultivars clearly influenced expression of the components of resistance. The latent period was longer, the pustule size was smaller, pustules per square centimeter of leaf area were fewer and r_G values were considerably lower for R-186 and Gudo. Therefore, the epidemic of the rust disease was both delayed and slowed down.

In spite of its susceptible reaction type, the moderately susceptible cultivar had a longer latent period and fewer pustules per unit leaf area than the susceptible cultivar, the combined effect of which resulted in a reduced AUDPC. This trait of the moderately susceptible cultivar is an expression of partial resistance (Parlevliet, 1979). High variability was observed among the cultivars for latent period, size and number of pustules per square centimeter leaf area. Several components, including these three, have been reported for other pathosystems (Parlevliet, 1975; Ohm and shaner, 1976; Habtu and Zadoks, 1995; Chongo and Bernier, 1999). This type of

resistance is quite common in other legume-rust pathosystems [e.g. in pea-*U. viciae-fabae* (Pal *et al.*, 1980), groundnut-*Puccinia arachidis* (Subrahmanyam *et al.*, 1993), chickpea-*U. ciceris-arietini* (Rubiales *et al.*, 2001), and faba bean-*U. viciae-fabae* (Sillero and Rubiales, 2002).

The cultivars tested varied for all components, disease severity and disease progress parameters (AUPDC, ADPC and r_G). The cvs. Gudo, R-186, and FLIP-87-66L, had longer latent period, and fewer and smaller pustules when compared with EL-142. Disease severities, APDC and AUDPC values also were lower on the four test cultivars than on EL-142. Three of these cultivars were effective in reducing the rate of the rust disease epidemic r_G, as well. FLIP-89-60L did not differ from EL-142 in infection type and r_G. However, FLIP-89-60L was different from EL-142 by having significantly longer latent period and fewer pustules. This is, perhaps, the reason why rust severity, APDC and AUDPC values were lower on this cultivar than on EL-142.

Of the three components investigated in the field, pustule size was the only component significantly correlated with the remaining two components, rust severity and with all disease progress parameters, particularly r_G. This means that pustule size is the most important component in deciding the apparent infection rate and thereby characterizing partial resistance in lentil. Apparently, this is contradictory to the conclusions made by Parlevliet (1979) in which he proposed that the latent period is the decisive component in the determination of the apparent infection rate r_G in polycyclic diseases such as the rusts. However, the latent period and pustule size are not independent. The strong correlation between the latent period and the pustule size ($r = -0.92$) obtained in this study provides evidence that these components are interdependent. Ohm and Shaner (1975; 1976) pointed out the possibility of linkage between genetic factors controlling these two components. The significant

correlations among the three components measured in the field suggest that the components were not independent. In other words, the components varied in association with each other. This is what Parlevliet (1979) refers to as an associated variation of the components of partial resistance. Such interdependence of the components was also detected in barley-leaf rust (Parlevliet, 1979) and lentil-anthracnose (Chongo and Bernier, 1999) pathosystems. The variation in the component of resistance latent period, does affect the variation in both pustule size and pustule density and eventually the variation in the epidemic development of lentil rust disease. Therefore, the more the number of the components used as selection criteria for resistance, the higher the levels of resistance would be in selected genotypes (Griffths and Jones, 1987; Chongo and Bernier, 1999).

Each component evaluated in the glasshouse was positively correlated with the same component in the field. Latent period, pustule size and spore production were significantly correlated with rust severity and AUDPC. This indicates that resistance evaluation in the glasshouse could be an alternative to resistance evaluation in field conditions.

Latent period was longer in the field than in the glasshouse for all cultivars except for EL-142. However, the ranking of genotypes between the glasshouse and the field was similar. Perhaps, the longer latent period observed in the field as compared with the glasshouse could be attributed to fluctuating weather conditions occurring in the field, especially temperature and leaf wetness, which influence this component. In alfalfa (*Medicago sativa* L.), latent period or the time to appearance of rust (*Uromyces striatus*) pustules is determined by post-inoculation temperature (Webb and Nutter, 1997). According to Webb and Nutter (1997), the time to first pustule appearance in alfalfa ranges from seven days for 30 °C to 21 days for 15 °C.

Chongo and Bernier (1999) reported latent period of anthracnose (*Colletotricum truncatum*) in lentil to be longer in the field than in the controlled environment.

Lentil genotypes significantly influenced the infection efficiency of *U. viciae fabae*. The infection efficiency varied from 0.055 on Gudo to 0.128 on EL-142. These two infection efficiencies indicate the ratios of pustules to spores were about 1 : 20 and 1 : 8 on Gudo and EL-142, respectively. A pustule to spore ratio of about 1 : 11 has been reported for *U. phaseoli* in pinto bean (Schein, 1964). The low infectibility of cv. Gudo could be partly explained by the high frequency of early aborted colonies and non-penetrations (see Chapter 7). In all other components studied in the glasshouse, test genotypes showed significant variations. Thus, the data on quantitative evaluation of the components will be useful for understanding the level of variation existing and eventually the effectiveness of selection.

On the bases of most of the components, final rust severity, AUPDC, ADPC and r_G values, Gudo and R-186 can be grouped together as having high level of resistance, with FLIP-87-66L being intermediate between this group and the susceptible EL-142 and the moderately susceptible FLIP-89-60L. It appears that the existence of a variable apparent infection rate and AUDPC values (from low to high) within the genotypes that have already been identified as susceptible, moderately susceptible, average reaction, moderately resistant and resistant, could be additional evidence for the presence of slow rusting in lentil for rust (*U. viciae-fabae*).

Even though values of rust severity, AUDPC and r_G do not perfectly match with the values of the resistance components in ranking the cultivars, they may be fairly easy parameters to compare and select genotypes with partial resistance under field conditions. Since there is correlation between rust severity and AUDPC, rust severity alone could also be used as a criterion for selecting genotypes with partial

resistance. In studies on slow rusting of faba bean (*Vicia faba* L.) to rust (*U. viciae-fabae*), Rashid and Bernier (1986) and Conner and Bernier (1982a) also suggested the use of final rust severity as a criterion for comparing and selecting lines possessing partial resistance in preliminary studies instead of AUDPC values, which require measuring rust severity many times in the season. From studies made on partial resistance of lentil to anthracnose (*Colletotricum truncatum*), Chongo and Bernier (1999) suggested the use of either AUDPC or rust severity values as selection criterion in the field, as well. The high correlation coefficient between APDC and rust severity merely indicate that the general assessment scheme for field ratings is adequate.

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Table 6.1 Effect of lentil genotypes on infection efficiency (IE), latent period (LP_{50}), spore production (SP) and pustule size (PS) of *Uromyces viciae-fabae* in a controlled environment

Genotype	IE[a]	LP_{50} (days)	SP (no. of spores/pustule)	PS (mm^2)
EL-142	0.128a	8.26b	2634a	0.562a
FLIP-89-60L	0.130a	8.32b	2161ab	0.493a
FLIP-87-66L	0.100b	8.44b	1563b	0.263b
R-186	0.080c	8.83a	892c	0.098c
Gudo	0.055d	9.00a	900c	0.096c
LSD .05	0.013	0.24	642	0.119
S.E. (±)	0.004	0.08	214	0.040
C.V. (%)	21	2	29	29

[a] Figures followed by the same letter in a column are not significantly different from each other at the 5% level of probability of the LSD test.

Table 6.2 Linear correlation coefficients among components of resistance to rust in lentils in the glasshouse

	IE[a]	LP_{50}	SP	PS
IE	—			
LP_{50}	-0.85*	—		
SP	0.73	-0.90*	—	
PS	0.82	-0.83	0.94**	—

*, ** Significant at 5% and 1% level of probability, respectively.
[a] IE, infection efficiency; LP_{50}, latent period; SP, spore production; and PS, pustule size.

Table 6.3 Lentil genotypes with respective area under the disease progress curve (AUDPC), apparent infection rate (*r*), pustule density (PD), area under the pustule density curve (APDC), latent period, pustule size (PS) and rust severity (RS) as measured in the field

Cultivar	AUDPC[a] (%-day)	r_G[b] (day^{-1})	PD[c] (no. cm^{-2})		APDC	Latent period (days)	PS[d]	RS (%)
EL-142	1682 a	0.081 a	2.28 a	(10.03)	114 a	8.25 c	3.38 a	65.35 a
FLIP-89-60L	839 b	0.075 a	1.11 b	(2.79)	56 b	15.50 b	3.35 a	54.68 b
FLIP-87-66L	532 c	0.056 c	0.96 b	(1.97)	48 b	15.25 b	2.50 b	37.50 c
R-186	264 d	0.049 c	0.37 c	(0.35)	19 c	20.00 a	2.00 bc	28.65 d
Gudo	37 e	0.018 d	0.23 c	(0.13)	12 d	21.00 a	1.40 c	7.30 e
LSD.05	123.7	0.019	0.30		15.27	3.57	0.75	8.67
S.E. (±)	80.3	0.013	0.19		9.91	2.32	0.48	5.63
C.V. (%)	12.0	19.8	19.5		19.9	14.5	18.6	14.6

[a]AUDPC, area under disease progress curve, (%-disease days). Figures followed by the same letter in a column are not significantly different from each other at the 5% level of probability of the LSD test.
[b] r_G, apparent infection rate.
[c] PD, pustule density (No. of pustules cm^{-2} leaf area). Data were subjected to square root transformation. Figures in parentheses are means of original values.
[d] PS, pustule size (measured on a 4-point scale, where 0 = no sign of infection, 1= uredinia of about 0.5 mm diam, 3 = uredinia of about 1 mm, 4 = uredinia > 1 mm) (Conner and Bernier, 1982b).

Table 6.4 Linear correlation coefficients for components of resistance, rust severity, apparent infection rate and area under the disease progress curve in five lentil cultivars infected with *Uromyces viciae-fabae* in the field at Akaki, Ethiopia, 2001

Component[a]	LP	PS	PD	APDC	RS	AUDPC	r_G
LP (days)	—						
PS (mm^2)	-0.92*	—					
PD ($no.cm^{-2}$)	-0.99**	0.93*	—				
APDC (PD-day)	-0.99**	0.93*	1.00	—			
RS ($\%.cm^{-2}$)	-0.90*	0.99**	0.90*	0.90*	—		
AUDPC (%-day)	-0.98**	0.95**	0.99**	0.99**	0.93**	—	
r_G (day^{-1})	-0.84	0.97**	0.84	0.84	0.99**	0.87*	—

[a] LP, latent period; PS, pustule size; PD, pustule density; APDC, area under the pustule density curve; RS, rust severity; AUDPC, area under the disease progress curve; r_G, apparent infection rate.
*, ** Significant at 5% and 1% level of probability, respectively.

Table 6.5 Linear correlation coefficients for components of resistance to lentil rust in the glasshouse and field

Glasshouse	Field						
	LP_{50}[a]	PD	ADPC	PS	RS	AUDPC	r_G
IE	-.072	0.73	0.73	0.92*	0.94**	0.79	-0.53
LP_{50}	0.86*	-0.84	-0.84	-0.97**	-0.95**	-0.84*	0.39
SP	-0.94**	0.95**	0.95**	0.91*	0.90*	0.94**	0.02
PS	-0.83	0.86*	0.86*	0.93*	0.91*	0.90*	0.03

*, ** Significant at 5% and 1% level of probability, respectively.

[a] LP, latent period; PS, pustule size; PD, pustule density; APDC, area under the pustule density curve; RS, rust severity; AUDPC, area under the disease progress curve; r_G, apparent infection rate.

Figure 6.1 Progress of rust over time on different lentil genotypes at Akaki, Ethiopia: Cultivars EL-142 (susceptible), FLIP-89-60L (moderately susceptible), FLIP-87-66L (with intermediate reaction), R-186 (moderately resistant), and Gudo or FLIP-84-78L (resistant).

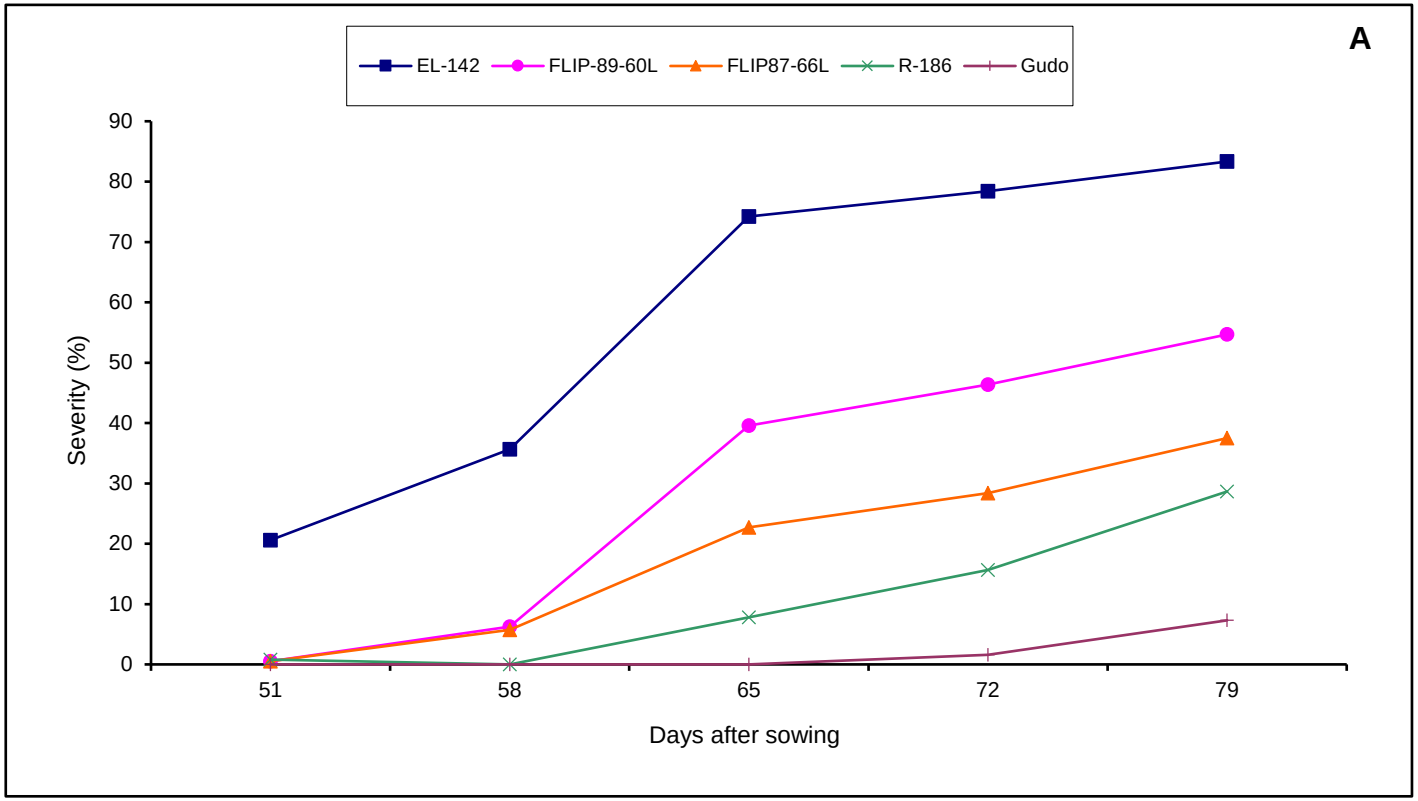
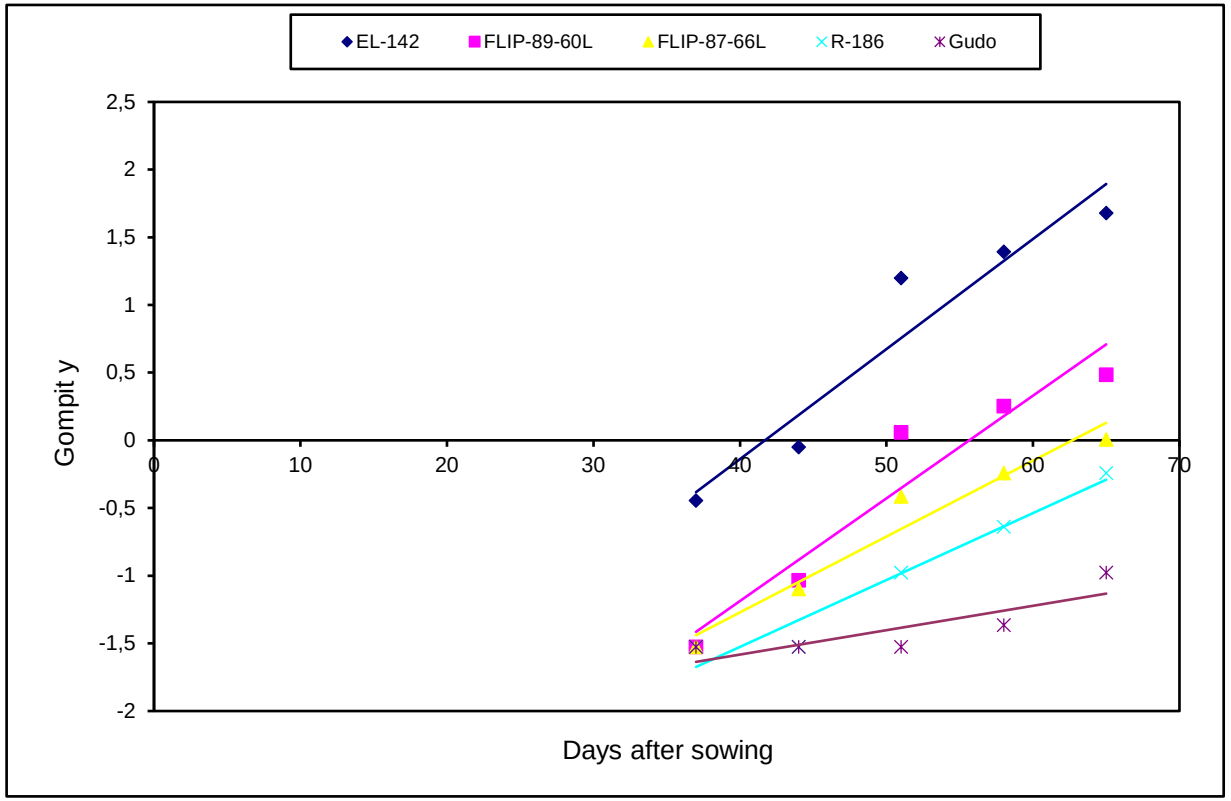

A
EL-142
FLIP-89-60L
FLIP87-66L
R-186
Gudo
Severity (%)
90
80
70
60
50
40
30
20
10
0
51
58
65
72
79
Days after sowing

Figure 6.2 Linearized progress of lentil rust on cultivars EL-142 (susceptible), FLIP-89-60L (moderately susceptible), FLIP-87-66L (average reaction), R-186 (moderately resistant), and Gudo (resistant).


EL-142
FLIP-89-60L
FLIP-87-66L
R-186
Gudo
2,5
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
0
10
20
30
40
50
60
70
Gompit y
Days after sowing

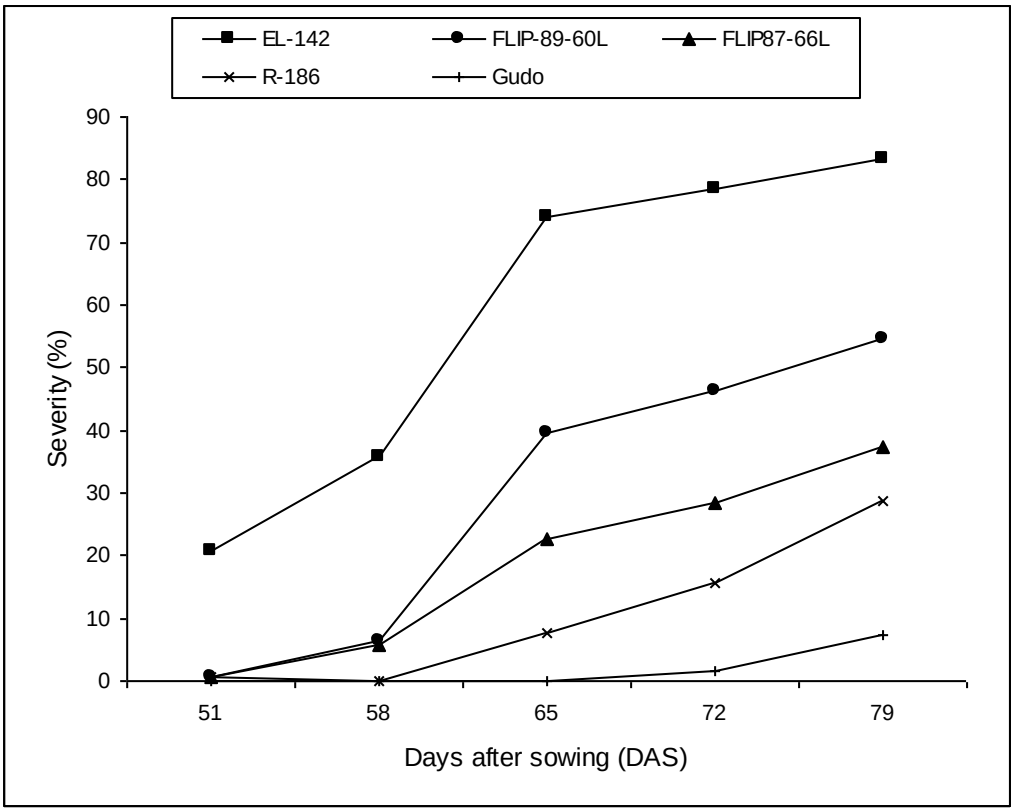

EL-142
FLIP-89-60L
FLIP87-66L
R-186
Gudo
Severity (%)
90
80
70
60
50
40
30
20
10
0
51
58
65
72
79
Days after sowing (DAS)

Figure 1.

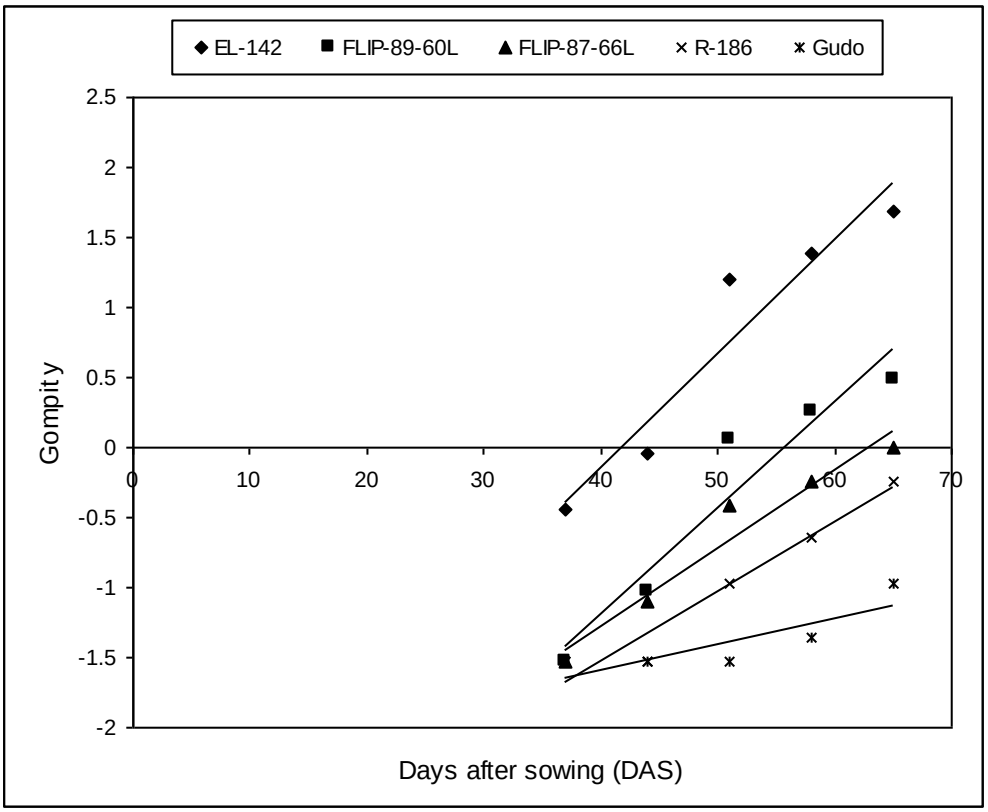

EL-142
FLIP-89-60L
FLIP-87-66L
R-186
Gudo
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Gompit y
0
10
20
30
40
50
60
70
Days after sowing (DAS)

Figure 2.

CHAPTER 7

HISTOLOGICAL CHARACTERIZATION OF RUST RESISTANCE IN LENTIL

"Histology is the study of cells and tissues at the microscopic level. Adjective: histological."

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ABSTRACT

Understanding host defence mechanisms and characterizing resistance at different levels of expression are important in the improvement of crop plants. To this end, histological reactions of two lentil cultivars, namely EL-142 (susceptible) and Gudo (resistant) to urediniospores of one mono-pustule isolate of (*Uromyces viciae-fabae*) were investigated by means of fluorescence microscopy. The histological factors studied included proportions of successful penetrations, early abortion, host cell necrosis and colony size. The percentage of germinated urediniospores that had not penetrated was significantly higher for the resistant cultivar Gudo than for the susceptible cultivar EL-142. Furthermore, Gudo had a higher percentage of early aborted colonies, frequently associated with host cell necrosis, than the susceptible cultivar. The host cell necrosis index for this cultivar was 1.13 indicating an excessive hypersensitive response to lentil rust. Resistance to rust in this cultivar was not of a complete hypersensitive type. At the macroscopic level, pustules were surrounded by chlorotic and necrotic flecks. Results of the present study indicate that the resistance in Gudo is due to prehaustorial and posthaustorial mechanisms. However, resistance to rust may not be durable due to its hypersensitive nature.

INTRODUCTION

Resistance in lentil (*Lens culinaris* Medik) to rust [*Uromyces viciae-fabae* (Pers.) J. Schröt] has been shown to be monogenically inherited (Sinha and Yadav, 1989; Singh and Singh, 1992). The simple inheritance of this resistance has facilitated its wide use in lentil resistance breeding programmes. This type of resistance, generally known as posthaustorial (hypersensitive) resistance (Niks and Dekens, 1991) may, despite the ease with which it is manipulated, have a significant impact on resistance breeding due to its ephemeral nature (Niks, 1982).

Histological research is useful to characterize quantitative resistance by microscopically studying the appearance of host cells and infectious structures. Little is known about the histology of infection of *U. viciae-fabae* in resistant and susceptible lentil genotypes. The present study was undertaken to characterise rust resistance in lentil seedlings inoculated with *U. viciae-fabae*.

MATERIALS AND METHODS

Inoculum multiplication

A pure culture of urediniospores of Akaki mono-pustule isolate 1 (AMPI1) of *U. viciae-fabae* was increased and maintained on the cv. EL-142 until inoculation.

Inoculation and incubation

Twelve-day-old plants (V6-V7 growth stage) (Erskine, Muehlbauer and Short, 1990) of cvs. EL-142 (susceptible) and Gudo (resistant) were inoculated in a settling tower (see Chapter 4). Each cultivar was replicated three times. Following inoculation, plants were

removed from the settling tower and placed in a dew chamber (relative humidity > 96% at 18-22°C under darkness for 24 h). Uninoculated plants of each cultivar were kept in the dew chamber to check if rust contamination had occurred before inoculation. Upon removal from the dew chamber, plants were placed, after drying-off, on a glasshouse bench at an approximate day/night temperature regime of 23/17ºC. Plants received natural daylight supplemented with illumination from cool-white fluorescent tubes, which supplied 120 μmol/m^2.s^{-1} for 14 h each day. Pots were arranged in a randomized complete block design.

Sample preparation, staining and microscopic examination

Four leaves (two bifoliates and two multifoliates) per cultivar were sampled from each replication at 5 days post-inoculation (d.p.i). Whole leaves were cleared and fixed in ethanol : dichloromethane (3 : 1 v/v) + 0.15% trichloroacetic acid for 24 h. Specimens were washed twice in 50% ethanol for 15 min, twice for 15 min in 0.05 M sodium hydroxide, and rinsed three times with distilled water before being submerged in 0.1 M tris[hydroxymethyl]aminomethane/ hydrochloric acid buffer (pH 5.8) and stained for 5 min in 0.1% diethanol (Uvitex 2B, Ciba-Geigy/Novartis/Syngenta, Basel, Switzerland) (Niks and Dekens, 1987) in the preceding buffer. This was followed by rinsing four times with distilled water and washing with 25% aqueous glycerol for 30 min. Stained leaves were stored in 50% glycerol containing a trace lactophenol to prevent deterioration of fungi and drying of material.

Observations on 150 infection sites per cultivar (50 infection sites on four leaf samples replicated three times) were carried out at 100x or 400x with a Nikon (Nikon

Corp. Tokyo, Japan) Optiphot epifluorescence microscope. The filter combinations UV-1A (excitation filter 330-380 nm and barrier filter 420 nm) for fungal structures, and B-2A (excitation filter 450-490 nm and barrier filter 520 nm) for observations of plant cell necrosis or autofluorescence measurements were used. Fungal structures fluoresced a bright light blue colour. Haustorium mother cells fluoresced extremely bright, whereas haustoria in the cell were not visible. Using the B-2A filter, host cells fluorescing an orange-yellow color were considered necrotic, whereas unaffected healthy cells did not fluoresce (Rohringer *et al*., 1977; Bender *et al.*, 2000). Only infection sites where appressoria had formed over stoma were considered as successful penetration attempts and accordingly studied to determine the proportion of sites where infection hyphae and haustorium mother cells occurred. All non-penetrated appressoria were considered as aborted penetration attempts. According to Niks (1982), a colony was considered "early aborted" if up to six haustorial mother cells were formed. The number of haustorial mother cells at each infection site was counted at 100 x magnification and confirmed at 400 x, where necessary.

Two dimensions of fungal colonies and the necrotic leaf area or host cell necrosis, if present, were measured with a calibrated eyepiece micrometer and corresponding areas (mm^2) calculated according to the formula: πr^2. A hypersensitivity index (Kloppers and Pretorius, 1995) was calculated by dividing the necrotic area by the corresponding colony size. The percentage of infection sites displaying host cell necrosis was also recorded. Genotypic differences were tested for significance using t-tests.

Infection type was scored 11 d.p.i. using the scale of Roelfs, Singh and Saari (1992) where 0 = no uredinia or other macroscopic sign of infection (immune), ; = no uredinia, but hypersensitive necrotic or chlorotic flecks present (nearly immune), 1= small uredinia surrounded by necrosis, 2 = small to medium uredinia often sourrounded by chlorosis or necrosis; green island may be sourrounded by chlorotic or necrotic border (moderately resistant), 3 = medium-sized uredinia that may be associated with chlorosis (moderately susceptible), 4 = large uredinia without chlorosis (susceptible).

RESULTS

Infection types 2 and 4 were recorded for cultivar Gudo and EL-142, respectively (Table 7.1). The proportion of non-penetrations was significantly ($P \leq 0.01$) higher in Gudo than in EL-142 (Table 7.1). Early abortion of colonies was significantly ($P \leq 0.01$) higher in Gudo than in EL-142. In Gudo, a high proportion of fungal colonies was associated with host cell necrosis. The hypersensitivity index calculated for this cultivar was 1.13. Infection units in EL-142 were rarely associated with necrosis. Differences were also found between the two cultivars in the size of the colonies (Fig. 7.1). Significantly ($P \leq 0.01$) smaller colonies were formed in Gudo than in EL-142. Colonies in EL-142 were eight times as large as those in Gudo.

DISCUSSION

Histological observations of leaves of EL-142 and Gudo revealed that resistance in Gudo was due to a combination of early aborted colonies, smaller colony size, abortive

penetration and host cell death. These suggest that defense reactions in Gudo were activated at the onset of and during the infection process.

Abortive penetration might be due to several factors acting singly or in combination against rust fungi. These include poor adhesion of germlings (germinating spore or germ tube) to the leaf surface (Mendgen, 1978; Wynn and Staples, 1981), deviation in micromorphology of the epidermal surface or surface/cuticular ridges that serves as cues in guiding the thigmosensing germ tubes toward stomates or triggering directional growth of germ tubes (Wynn and Staples, 1981) and morphology of stomatal guard cells (Wynn, 1976). Rubiales *et al.* (2001) reported the leaf wax layer (the wax covering of the stomatal apparatus) to play a role in the exclusion of *Puccinia hordei* prior to stomatal penetration of *Hordeum chilense* leaves. The existence of such morphological traits and their role in excluding the rust fungus from entering the stomates in lentil are yet to be confirmed.

In addition to the high levels of early aborted colonies in Gudo, infection hyphae were frequently accompanied by cell necrosis which was expressed macroscopically on the leaves by an infection type 2. According to Dixon, Harrison and Lamb (1994), resistance in fungal-plant interactions is often associated with the hypersensitive resistance in which a limited number of cells die rapidly when they come in direct contact with the invading pathogen. Recently, Sillero and Rubiales (2002) indicated that early abortion could be related to host cell necrosis. Clearly, necrotic reaction acts against biotrophs such as *U. viciae-fabae* that depend entirely on living host cells for essential material. In general, necrosis plays an important role in resistance where the rapid death of the invaded cell is followed by no further fungal growth (Heath, 1972). On the other

hand, hypersensitivity may merely be a consequence or side effect of the rust resistance mechanisms (Kowalska and Niks, 1999) or visible product of a number of previous interactions between a host and the pathogen (Heath, 1976). Although much remains to be done regarding the mechanism controlling the hypersensitivity in plant/pathogen interaction, it is apparent that hypersensitive resistance is accompanied by biochemical changes both at the site of infection and at distant sites in the plant leading to local and systemic gene activation and eventually to the disease resistance phenotype (Kombrink and Schmelzer, 2001).

Data from this study clearly indicate that resistance of Gudo to rust is incomplete because some cells were infected and it was of a hypersensitive nature. This is different from the incomplete hypersensitive type of resistance occurring in flax to flax rust (Kowalska and Niks, 1999) and in faba bean to *U. viciae-fabae* (Sillero and Rubiales, 2002). Incomplete hypersensitivity reported by Kowlska and Niks (1999) refers to the cases where hypersensitivity is expressed late in the infection process and only by some infected plant cells. In case of Gudo, however, the expression of hypersensitive reaction was observed as early as 24 h post-inoculation.

In resistance studies, the use of fluorescence microscopy and digital image technology could help facilitate studies of host-parasite interaction in the lentil-rust pathosystem. Rubiales and Sillero (2003) have used the technique successfully in investigating microscopic components of faba bean resistance to rust (*U. viciae-fabae*). Information generated from histological studies should provide a better understanding of the nature of lentil rust resistance.

The cultivar Gudo was identified and developed at the Debre Zeit Agricultural Research Center (DZARC) by testing it at many locations repeatedly (Bejiga, Million and Yadeta, 1995). This test protocol coupled with the slow rate of rust development on this cultivar observed in resistance study (see Chapter 6) could be indications of the stability of rust resistance in this cultivar. However, neither testing at many locations nor experimental data on slow disease development is necessarily a proof of durable resistance (Johnson, 1984). Although Gudo was shown to have partial resistance to rust, it may not be durable due to the hypersensitive nature revealed in this study.

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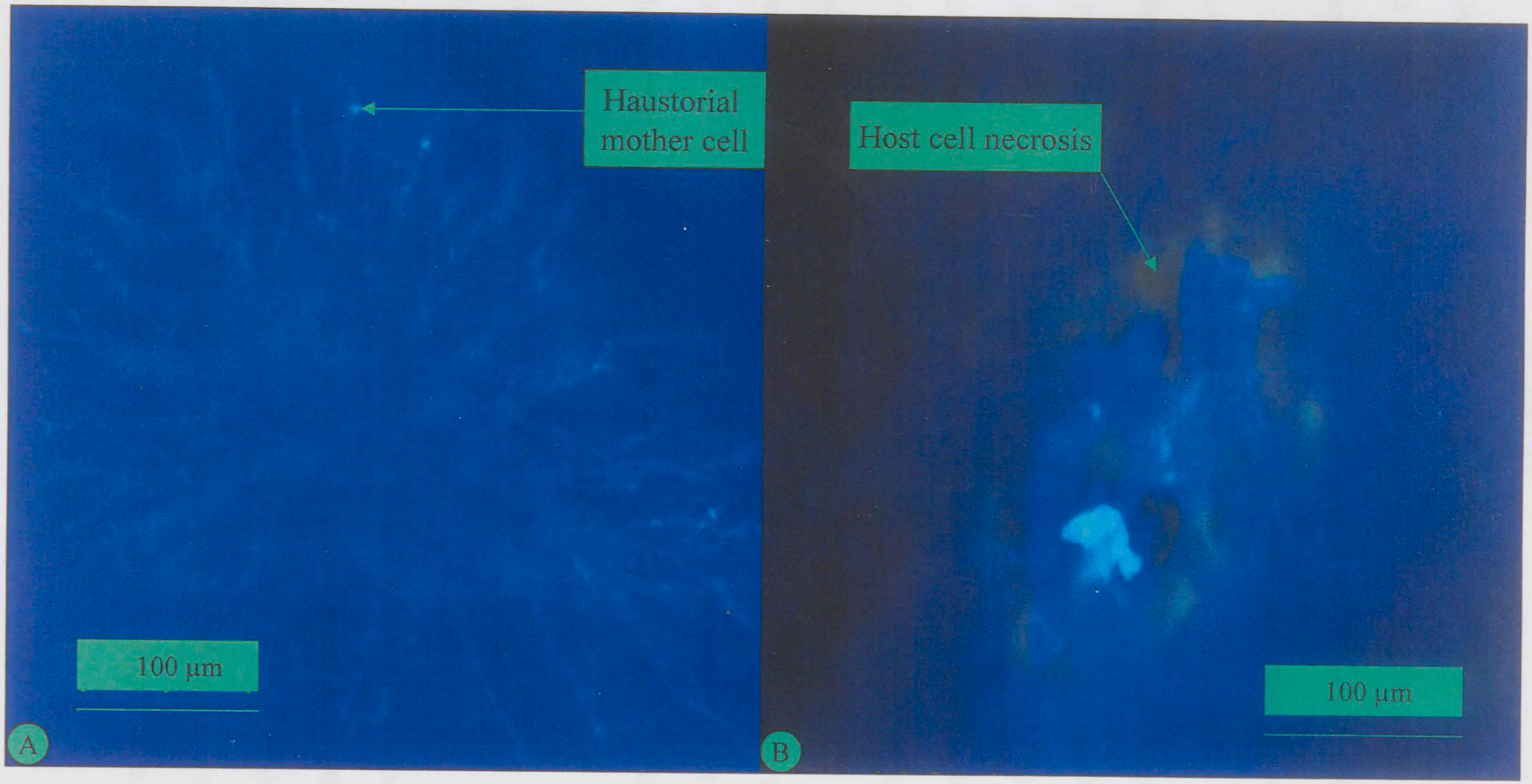
Table 7.1 Colony size, number of early aborted colonies and proportions of non-penetrated germ tubes of *Uromyces viciae-fabae* in lentil cultivars EL-142 (susceptible) and Gudo (resistant) 5 days post-inoculation

Genotype	Histological observations					Infection type
	Colony size (mm^2)*	Proportion of non-penetration	Proportion of early abortion	Infection sites with host cell necrosis	Hypersensitivity index	
EL-142	0.161a	0.06b	0.00b	0.00	0.00b	4
Gudo	0.02b	0.27a	0.56a	91.86	1.13a	2

* Values within each column followed by the same letter are not statistically different from each other according to a one-tailed t-test at a 0.05 level of significance. Each value in a column is a mean of 150 infection sites.

Figure 7.1 Rust colony size (5 days post-inoculation) in leaf tissue of seedlings of (A) susceptible lentil cultivar EL-142 and (B) resistant Gudo.


Haustorial
mother cell
100 µm
A
Host cell necrosis
100 µm
B

SUMMARY

Rust [*Uromyces viciae-fabae* (Schroet.)] is one of the major diseases of lentil (*Lens culinaris* Medik.) in the world causing major crop losses when conditions are conducive for disease development. Effective lentil rust management depends on knowledge of, e.g., disease - yield relationships, dynamics of rust epidemics, accurate phenotyping of resistance, and components of resistance.

Sequential analyses of lentil rust epidemics were performed by assessing crop and disease parameters in five field rust epidemics. The five epidemics produced similar-shaped disease progress curves that varied significantly in the rate of temporal progression (r_L) and area under the disease progress curve (AUDPC). The epidemics did not affect crop growth; however, they had significant influences on seed yield, pods per plant and seed mass at crop growth stages between early flowering and early pod formation. The rust also significantly affected harvest index and days to maturity. Results demonstrated that a seed yield loss of up to 41.7% could occur and the effect of rust on seed yield of lentil can be predicted with AUDPC and critical-point models using disease severity assessed on the upper canopy layer in the early flowering stage. Every 1% increase in rust severity reduced seed yield by 8.39%. Rust severity ≥ 4.7% at the critical stage will significantly reduce seed yield.

To assess the effect of rust on the value of infected lentil straw as animal feed, a trial was conducted to evaluate its degradability in rumens of Zebu cows. Following 6 h of incubation, samples of all treatments lost ≥ 40% of the total dry matter, degradation reaching ca. 65% at 72 h. Rust did not decrease degradability of dry matter in the rumen. The nitrogen (N), phosphorus (P) and the total crude protein (TCP) content in rust

infected straw was higher than those of healthy straw. Seeds from healthy plants contained more P than the seeds from rusted plants. The rust had no effect on seed N content.

Inoculation and incubation techniques resulting in uniform spore deposition and infection, thereby obtaining reproducible and accurate data on host responses, are required for quantitative studies. To achieve this, a spore-settling tower was developed, and uniformity of spore deposition was assessed by dispersing 1, 2, 4 and 8 mg urediniospores of *U. viciae-fabae* into the tower. Uniform spore deposition was obtained when 8 mg spore quantity was discharged into the tower and allowed a settling period of 3 min. A linear relation was found between mass of spores discharged into the tower and spores deposited/cm^2. Uniformity of spore deposition increased as the spore amount discharged increased.

In experiments quantifying the effects of temperature on germination of *U. viciae-fabae*, a high percentage (≥ 80%) of spore germination was observed after 3 h of incubation on 1.5% water agar at 10, 15, 20 and 25°C, with an optimum (99%) at 20°C. At this sampling time the length of germ tubes ranged from 66 µm (10°C) to 196 µm (20°C). For minimum infection of lentil cultivar EL-142 at 20°C, a dew period of at least 3 h was required, whereas maximum infection occurred with a dew period of 24 h.

Components of resistance to *U. viciae-fabae*, namely, latent period, infection efficiency, pustule size and spore production were evaluated in the lentil cultivars Gudo, R-186, FLIP-87-66L and FLIP-89-60L and EL-142 (susceptible check) in a glasshouse. The cultivars were also compared for area under the disease progress curve (AUDPC), area under the pustule density curve (APDC), apparent infection rate (r_G), and disease

severity under field conditions. Gudo and R-186 had significantly smaller and fewer pustules, lower spore yield and longer latent period than EL-142. FLIP87-66L was intermediate for infection efficiency and pustule size. In addition, significant differences were found between cultivars for AUDPC, APDC, disease severity and r_G. Estimates of AUDPC, APDC, disease severity and r_G were reduced in Gudo, R-186 and FLIP-87-66L compared with the susceptible check EL-142. FLIP-89-60L also showed low AUDPC, APDC and disease severity. Some of the components obtained in the field were significantly correlated with each other and those measured in the glasshouse. Most of the components studied in the glasshouse were correlated with AUDPC and disease severity. Data indicated the existence of incomplete (partial) resistance in the test cultivars, and the possibility of using infection efficiency, latent period, spore production and pustule size as selection criteria in the evaluation of partial resistance to rust in lentil. Since there was an interdependence of the components, selection based on more than one component helps obtain lines with higher levels of partial resistance. The AUDPC, disease severity and r_G could also be used for selecting lines with partial resistance in the field. Histological studies showed that the resistance mechanism in the lentil cv. Gudo against *U. viciae-fabae* is a combination of hypersensitive and pre-penetration types. Furthermore, cv. Gudo had a higher percentage of early aborted colonies than the susceptible cultivar.

OPSOMMING

Roes [*Uromyces viciae-fabae* (Schroet.)] is een van die belangrikste siektes van lensies (*Lens culinaris* Medik.) wêreldwyd en kan totale oesverliese onder gunstige toestande veroorsaak. Doeltreffende roesbeheer berus op kennis van, onder andere, siekte – opbrengsverwantskappe, dinamika van roesepidemies, akkurate fenotipering van weerstand en komponente van weerstand.

Opvolgende analises van lensieroes is uitgevoer ná beraming van gewas- en siekteparameters in vyf veldepidemies. Die epidemies het vorderingskurwes met ooreenstemmende vorm, maar verskillende tempo's (r_L) en area onder die siektevorderingskurwe (AOSVK) tot gevolg gehad. Die epidemies het nie gewasgroei beïnvloed nie, maar wel saadopbrengs, peule per plant en saadmassa tussen vroeë blom en vroeë peulvorming. Roes het ook oesindeks en dae tot oesrypheid beduidend beïnvloed. Resultate het getoon dat saadmassa met tot 41.7% verminder kan word. Voorts kan die effek van roes op lensiesaadmassa voorspel word met AOSVK en kritiese-punt modelle wat gebruik maak van siektegraad in die boonste blaredak tydens vroeë blom. Elke 1% toename in siektegraad het saadopbrengs met 8.39% verminder. Roesinfeksievlakke ≥ 4.7% tydens die kritiese groeistadium sal saadmassa dus betekenisvol verminder.

Ten einde die effek van roes op dierevoedingswaarde van geïnfekteerde lensiestrooi te bepaal is 'n eksperiment gedoen om die afbraak daarvan in Zebu-koeie te meet. Na 'n 6 h inkubasieperiode het alle monsters ≥ 40% van die totale droë materiaal verloor, met 'n verlies van sowat 65% na 72 h. Roes het nie die afbreek van droë materiaal in die rumen verlaag nie. Die stikstof (N)-, fosfor (P)- en totale ru-

proteïeninhoud (TRP) van roes-geïnfekteerde strooi was hoër as in gesonde strooi. Saad van gesonde plante het meer P bevat as dié van roesplante. Roes het geen effek op die N-inhoud van saad gehad nie.

Inokulasie- en inkubasietegnieke wat uniforme spoordeponering en infeksie tot gevolg het is noodsaaklik vir herhaalbare en akkurate gasheerdata in kwantitatiewe studies. Om hierdie doelwit te bereik is ‘n spoor-neerleggingstoring ontwikkel en die deponering van 1, 2, 4 en 8 mg urediniospore van *U. viciae-fabae* is bepaal. Uniforme plasing is verkry wanneer 8 mg spore in die toring vrygestel en toegelaat is om vir 3 min uit te sak. ‘n Liniêre verwantskap is verkry tussen massa en die hoeveelheid spore gedeponeer/cm^2. Uniformiteit het verbeter namate die hoeveelheid vrygestelde spore toegeneem het.

Tydens die kwantifisering van temperatuureffekte op die ontkieming van *U. viciae-fabae* is ‘n hoë persentasie (≥ 80%) spoorontkieming na 3 h inkubasie op 1.5% water agar by 10, 15, 20 and 25°C, met ‘n optimum (99%) by 20°C, gevind. By hierdie monsteringstyd het die kiembuislengte gewissel tussen 66 µm (10°C) en 196 µm (20°C). Vir infeksie van lensiekultivar EL-142 by 20°C was ‘n minimum douperiode van 3 h nodig. Maksimum infeksie het voorgekom na ‘n 24 h douperiode.

Komponente van weerstand nl. latente periode, infeksiedoeltreffendheid, puisiegrootte en spoorproduksie is in ‘n glashuis gemeet vir die lensiekultivars Gudo, R-186, FLIP-87-66L, FLIP-89-60L en EL-142 (vatbare kontrole). Die kultivars is ook vergelyk vir AOSVK, area onder die puisiedigtheidskurwe (AOPDK), infeksietempo (r_G) en siektegraad onder veldtoestande. In vergelyking met EL-142 het Gudo en R-186 betekenisvol kleiner en minder puisies gehad, asook laer spoorproduksie en ‘n langer

latente periode. FLIP87-66L was intermediêr vir infeksiedoeltreffendheid en puisiegrootte. Betekenisvolle verskille is gevind tussen kultivars vir AOSVK, AOPDK, siektegraad en r_G en hierdie komponente was laer in Gudo, R-186 en FLIP-87-66L as EL-142. FLIP-89-60L het ook lae AOSVK, AOPDK en siektegraad getoon. Sommige van die komponente in die veld gemeet het betekenisvol gekorreleer met mekaar asook met glashuiskomponente. Meeste komponente in die glashuis gemeet het gekorreleer met AOSVK en siektegraad. Data het die teenwoordigheid van onvolledige (gedeeltelike) weerstand in die kultivars aangedui met die moontlikheid om infeksiedoeltreffendheid, latente periode, spoorproduksie en puisiegrootte as seleksiemaatstawwe te gebruik. Aangesien ‘n interafhanklikheid van komponente voorgekom het kan seleksie vir meer as komponent help om lyne met hoër vlakke van gedeeltelike weerstand te ontwikkel. AOSVK, siektegraad en r_G kan ook vir seleksie van gedeeltelike weerstand in die veld gebruik word. Histologiese studies het getoon dat die weerstandsmeganisme teen *U. viciae-fabae* in die lensiekultivar Gudo ‘n kombinasie is van hipersensitiewe en prepenetrasie tipes. Gudo het ook ‘n hoër persentasie vroeë aborsies as EL-142 gehad.