THE BIOLOGY AND CONTROL OF WILT OF CHICKPEA CAUSED BY *FUSARIUM OXYSPORUM* f. sp. *CICERI*

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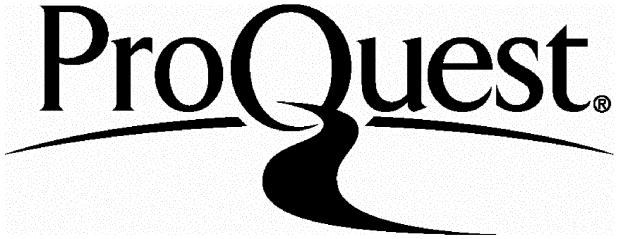

This thesis is dedicated to the memory of my father

Sadok Halila

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ABBREVIATIONS

Standard abbreviations are used throughout the text, they are the following:

WANA - West Asia and North Africa.

FAO - Food and Agricultural Organisation of the United Nations, Rome, Italy.

ICARDA - International Centre for Agricultural Research in the Dry Areas, Syria.

ICRISAT - International Crop Research Institute for the Semi-Arid Tropics, India.

INRAT- Institut National de la Recherche Agronomique de Tunisie (Tunisian Agricultural Institute)-Tunisia.

FLIP - Food Legume Improvement Program - ICARDA.

ID - Inoculum Density.

FOC - *Fusarium oxysporum* f. sp. *ciceri.*

LM - Liquid Medium

LM+CE- Liquid Medium + Chickpea Extract.

CSM - Chickpea Seed Medium.

WSP - Wilt sick plot.

ILC - International Legume Chickpea.

ABSTRACT

Fusarium oxysporum f.sp.*ciceri* (FOC) was isolated from 246 chickpea plants showing symptoms typical of *Fusarium* wilt. Another five plants yielded *Fusarium solani*. FOC isolates were classified into three groups, FG_3, F/SG_3 and FG_4 on the basis of mycelial morphology, radial growth rate and conidial dimensions. All isolates belonged to race 0 as determined by their reaction on 16 chickpea varieties.

The effect of a range of inoculum densities (500, 1000, 2000 and 4000 colony forming units/gram of soil) in combination with five air temperature régimes (10, 15, 20, 25 and 30°C) on wilt incidence was studied in three susceptible chickpea varieties, ILC 223, ILC 482 and ILC 3279. The incidence of wilt increased with increasing inoculum levels and with increasing temperature up to 25°C.

Kabuli chickpea varieties were screened in a wilt sick plot (WSP) and rated as susceptible when wilt incidence was >10%. The 1,915 varieties screened were placed in four classes on the basis of the timing of the onset of wilt symptoms. These developed within a 2 week period in all four classes which were described as very early wilters, early wilters, late wilters and very late wilters depending on whether >10% plants showed symptoms 28, 42, 56 or 70 days after emergence. In a further class disease incidence

increased slowly throughout the growing season. Resistance was found in 110 varieties and these maintained their resistance in a laboratory screening procedure.

Environmental variables most closely associated with rapid disease increase were high maximum daily temperature and cumulative numbers of days in which the mean temperature was >25°C.

Preliminary studies to determine the mechanism by which FOC causes wilt symptoms showed that culture filtrates of isolate FG_3 and Spanish race 0 were toxic to leaf cells isolated from the plant. Maximum toxic activity was obtained when isolate FG_3 was grown on chickpea seed medium for 2 weeks.

Field management of FOC disease was achieved by planting chickpea varieties, which combined the characters of late wilting and early flowering, in winter so that growth of the plant was essentially complete by the time temperatures became favourable for disease development.

CHAPTER I

INTRODUCTION

1.1. THE CHICKPEA: THE CROP AND THE PLANT

Chickpea (*Cicer arietinum* L.) belongs to the family *Leguminosae* which is second in size to the *Gramineae* (Aykroyd and Doughty, 1964). It is a food legume as distinct from oil-bearing and pasture or forage legumes. Food legumes are eaten as mature dry seeds, in which form they are usually known as "pulses" or as green pods which contain the immature seeds. The dry seeds may be boiled, grilled, baked or milled.

Chickpea, as a pulse crop, is an important source of protein for the people of the Indian subcontinent, West Asia and North Africa (WANA) regions (Saxena, 1989). It probably originated in the area of present-day south eastern Turkey and neighbouring northern Syria (Van der Maesen, 1972) and has spread to other geographical regions of the world because of the versatility of its adaptation to different environments. As a result it is known by a variety of names according to the local language including pois chiche (French), homos (Arabic), chickpea (English), grao-de-bico (Portugese), garbanzo (Spanish), chana (Hindi, Punjabi, Urdu) and kadalai (Tamil).

Chickpeas have been divided into two broad groups, "kabulis" and "desis" based on seed colour, shape and to some extent on seed size (Muehlbauer *et al.*, 1982; Van der Maesen, 1987). "Kabuli" types have white flowers which are

devoid of anthocyanin pigmentation. Stems and seeds also lack anthocyanins. This group is grown in the Mediterranean area, the Near East and Central and South America. They account for 10 to 15% of the world's chickpea production. "Desi" are small-seeded, irregularly shaped and normally dark-coloured. Flowers and stems usually have anthocyanin pigmentation. "Desi" types predominate in the Indian subcontinent, East Asia, Iran, and Afghanistan and account for 85-90% of world production.

Chickpeas have a diploid chromosome number of 16. They are branching annuals with an erect or spreading habit, and a height varying between 25 and 60 cm. The plant has a taproot that is 60 to 200 cm long. Leaves are either pinnately compound with 3 to 8 leaflet pairs or, less commonly, have a unifoliate structure. The self-pollinated flowers are typically papilionaceous and usually 0.6 - 1.3 cm long. They are borne in doubles or triples on inflorescences that originate from the stem axes. Flower colour is usually white for the "kabuli" types and can be either white, pink, purplish or blue for the "desi" types. The pods are rhomboid - ellipsoid about 1.9-5.1 cm long with an inflated appearance and glandular pubescence. They contain 1-2 seeds (sometimes up to 3) with smooth or rough coats.

1.2. AGRICULTURAL IMPORTANCE OF CHICKPEA

Chickpea is the third most important pulse crop in the world after dry beans (*Phaseolus vulgaris* L.) and dry peas

(*Pisum sativum* L.). It is grown in 33 countries on an average annual area of nearly 9.5 million hectares (Jodha and Subba Rao, 1987; FAO, 1988). Chickpea has a rotational role with cereals in various cropping systems, especially those used in the WANA region. The plant has the ability to fix atmospheric nitrogen when inoculated with appropriate strains of *Rhizobium* and, hence, improves soil fertility for the subsequent cereal crop (Papastylianou, 1987; ICARDA, 1981).

Chickpea is a particularly important pulse crop in North Africa and is ranked second after faba bean (*Vicia faba* L.) in the three countries of the Maghreb region which comprise Tunisia, Algeria and Morocco. (Nassib *et al.*, 1986; Halila *et al.*, 1988; Benbelkacem, 1988; Bounejmate, 1988). In Tunisia, in particular, chickpea is an important component of pulse production, representing 30% of the total (Halila *et al.*, 1988). All the chickpea produced in North Africa is of the "kabuli" type and accounts for about 67% of world "kabuli" production (Saxena, 1989). Yields of chickpea in North Africa fluctuate but the trend is downward (Halila *et al.*, 1988; Benbelkacem, 1988 and Bounejmate, 1988). The causes of fluctuation and limitation of yield can be grouped in three major categories: environmental, agronomic and biotic (Saxena, 1989).

Chickpea in WANA, hence in Tunisia, is traditionally grown as a spring-sown crop on soil moisture conserved from winter rainfall but it matures in the early summer when it is usually exposed to terminal drought and heat stress.

Agronomic factors that limit yield are insufficient use of fertilisers, herbicides, inadequate preparation of the land and inefficient seeding methods. However, it is now believed that biotic factors may be the most important cause of yield reduction and instability. These include diseases such as *Ascochyta* blight [*Ascochyta rabiei* (Pass.) Labr.], wilt caused mainly by *Fusarium oxysporum* Schlecht. emend. Snyd. and Hans. f.sp. *ciceri* (Padwick) Snyd. and Hans., root rots, stunts, nematodes, insect pests and parasitic weeds.

1.3. WILT AND ROOT ROT DISEASES OF CHICKPEA

Diseases are a major production constraint. More than 70 pathogens have been reported, so far, on chickpea from different parts of the world (Nene *et al.*, 1984). Among these, wilt and root rots are widespread and economically important wherever the crop is grown (Nene, 1979). At one time they were thought to form a disease complex (Nene *et al.*, 1978) but later Nene *et al* (1981) concluded that they were distinct, diagnosable diseases. Of these, chickpea wilt, caused by *Fusarium oxysporum* Schlecht. emend. Snyd. and Hans. f.sp. *ciceri* (Padwick) Snyd. and Hans. hereafter designated as FOC, is probably the most damaging and the most widespread since it has been reported from 23 countries (Nene *et al.*, 1989). In the Mediterranean basin, Jiménez-Diaz *et al.* (1989c) concluded, after extensive surveys, samplings and isolations, that the most prevalent and damaging chickpea disease in southern Spain is *Fusarium*

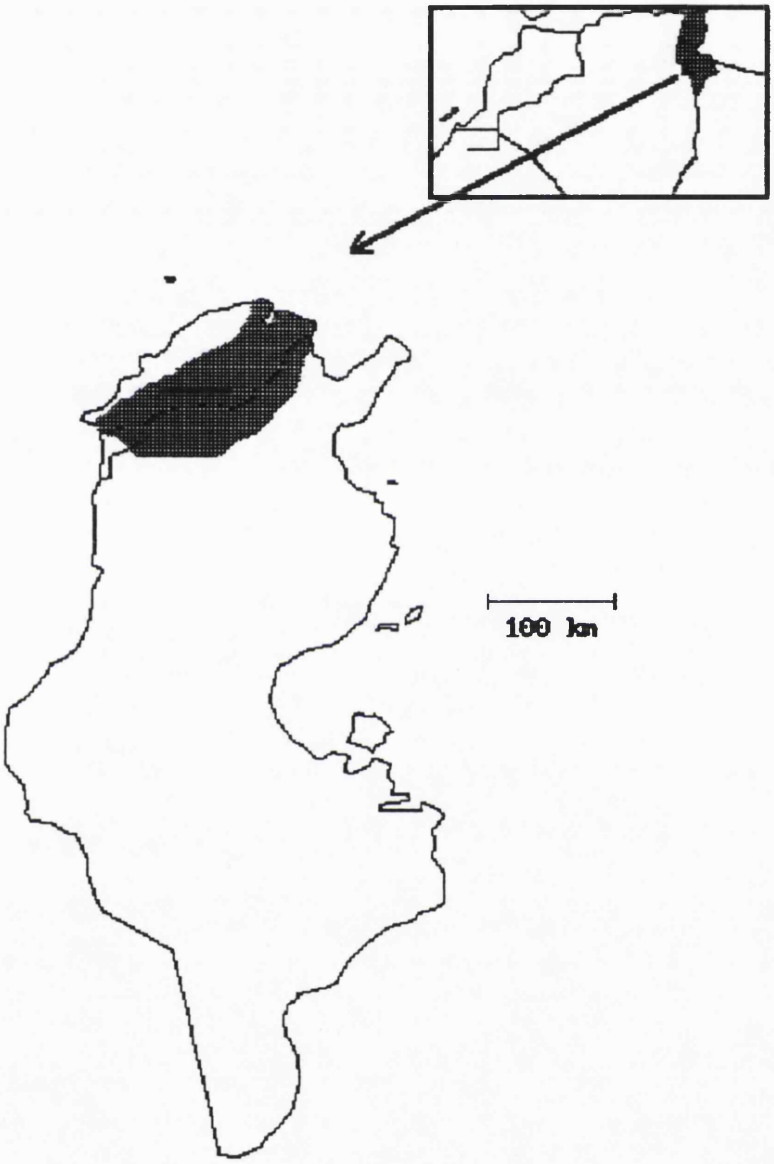
wilt with an annual yield loss of 12 - 15%. Similarly, in North Africa, *Fusarium* wilt was also reported to be a major chickpea disease in Tunisia (Halila *et al.*, 1984; Halila and Harrabi, 1990), Algeria and Morocco (Haware, 1990). Losses of up to 70% have been reported to occur in some years in Northern India and Pakistan (Punjab) (Grewal and Pal, 1970), while Singh and Dahiya (1973) reported an overall loss of 10% for India. Moreover, the production of "kabuli" chickpeas in California has declined in recent years, mainly because of wilt (Buddenhagen and Workneh, 1988).

In Tunisia, as well as in the other Maghreb countries, *Fusarium* wilt was not properly diagnosed until recently as the disease was often masked by the foliar pathogen, *Ascochyta rabiei*. Blight, caused by this fungus appears early in the season, while *Fusarium* wilt generally appears later. However, seasons that are favourable to *Ascochyta* blight are not usually favourable to *Fusarium* wilt and vice-versa.

Since 1985, regular surveys in Tunisia have revealed the importance of the disease in the chickpea growing areas. It is estimated that 30-40% of the fields in the chickpea belt are infested by *Fusarium* wilt and losses may be total (Fig. 1; Halila, unpublished data). Similarly, regional surveys in Algeria and Morocco on food legume problems organised by ICARDA in the region have shown the potential danger of this disease in eastern Algeria and northern Morocco but figures for losses are not yet known

Fig. 1:

Chickpea production zones (shaded) in northern Tunisia.


100 km

(Haware, 1990; Halila, Personal observations). Being soil and seed borne, FOC can survive in the soil for more than 6 years (ICRISAT, 1985) and spread by means of infected seeds (Haware *et al.*, 1978). Because of this, scientists in North Africa forsee *Fusarium* wilt as becoming the most important disease of this crop (S.P.S. Beniwal, M. Solh, M.P. Haware; personal communication). The work reported in this thesis is a contribution to the understanding of the chickpea-*Fusarium* wilt relationship.

1.4. *FUSARIUM* WILT OF CHICKPEA

1.4.1. History

The disease was first described in India in 1918 and the causal organism was named *Fusarium orthoceras* var. *ciceri* in 1940 by Watts Padwick (Van der Maesen, 1972 and Kraft *et al.*, 1986). Following the classification of Snyder and Hansen, the pathogen was renamed *Fusarium oxysporum* f.sp. *ciceri* (Chattopadhyay and Sen Gupta, 1967 and Booth, 1971, 1977). Work on *Fusarium* wilt was first initiated in India by Watts Padwick and Prasad (Van der Maesen, 1972) and most research has been done on the "desi" type. Later, ICRISAT joined the research effort and has become a reference centre for the disease. In the Mediterranean basin, the disease was first reported by Benlloch (1941, 1949). Since then, limited research has been done on soilborne fungi that infect chickpea until recently (Trapero-Casas, 1983; Trapero-Casas and Jiménez-Diaz, 1983, 1984, 1985a, b; Halila *et al.* 1984; Cabrera de la Colina

et al., 1985; Gonzales Torres, 1985; Cabrera de la Colina, 1986; Halila and Harrabi, 1990).

1.4.2. The fungus

The fungus belongs to the genus *Fusarium* , Class *Septomyces* and Subclass *Adelomycetes* (Booth,1971). Van der Measen (1972) described the fungus as having a whitish mycelium with a pigmented plectenchymatous layer in some isolates. Ovoid microconidia and spindle-shaped macroconidia with several septa are present and sometimes solitary one- or two-celled chlamydospores occur either in a terminal or intercalary position. However, tremendous variation in cultural and morphological characters of *Fusarium* isolates that caused wilt in chickpea were observed (Chattopadhyay and Sen Gupta, 1967 and Watts Padwick, 1940). Chattopadhyay and Sen Gupta (1967) studied the cultural variation of 20 monoconidial isolates and gave detailed descriptions of their conidial and cultural characters on three different media, namely potato dextrose agar, oat meal agar and steamed rice. The implications of their results for the taxonomic position of *Fusarium* were also discussed.

The cultural variation of *Fusarium oxysporum* has been highlighted by several workers (Snyder and Hansen, 1940; Messiaen and Cassini, 1968; Toussoun and Nelson, 1968, 1976; Nelson, 1981) and this variability caused Snyder and Hansen (1940) to advocate a new species concept. This is a modification of the Wollenweber and Reinking (1935)

system in which all species in the section *elegans* are combined into one species, namely *Fusarium oxysporum* and pathogenicity for specific hosts was used as a criterion for creating formae speciales.

The best description of asexual spore production by formae speciales of *Fusarium oxysporum* Schelt. emend. Snyd. and Hans, including FOC, was given by Nelson (1981). He distinguished three types:

1. Macroconidia produced most often on branched conidiophores in sporodochia, but may also be produced singly on aerial mycelium in culture. They are thin-walled, 2-5 septate, with a definite foot cell and a pointed apical cell.
2. Microconidia which are kidney-shaped and occur on short microconidiophores.
3. Chlamydospores which are thick-walled and are produced in hyphae or conidia, through condensation of their contents. They are formed both in culture and in dead host plant tissue in the final stages of wilt and can survive for an extended time.

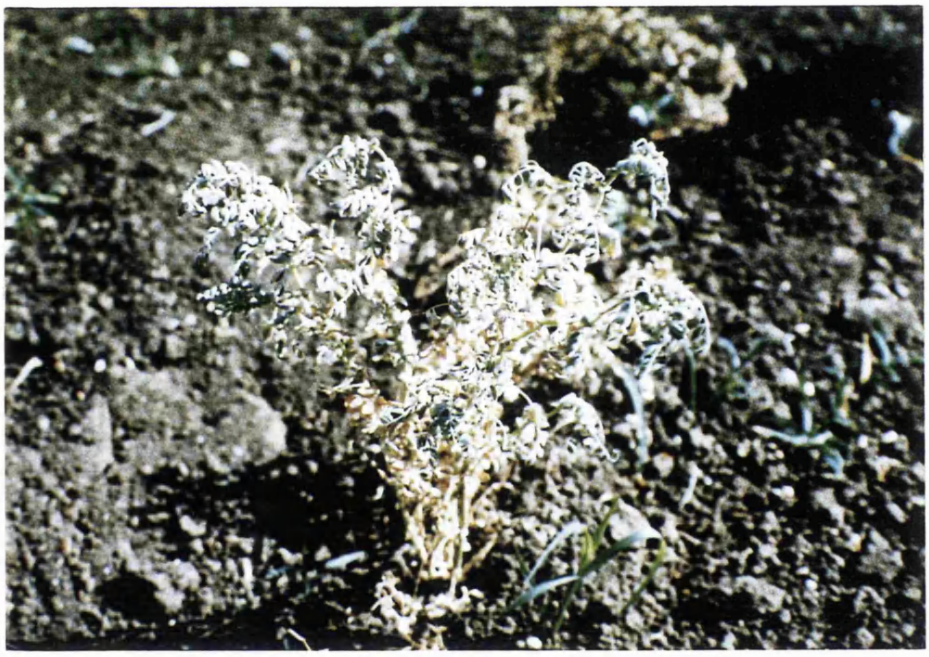
1.4.3. Symptoms

FOC may be diagnosed on the basis of field symptoms which characteristically include:

1. Sudden drooping of leaves and petioles which may be associated with yellowing (Fig. 2).
2. Discoloration of xylem and pith with colonization of the vessels by the fungal hyphae (Van der Maesen,

Fig. 2:

***Fusarium* wilt symptoms on chickpea plant** : Note the drooping of the leaves and the plant showing a "thirsty" appearance.



1972; Nene *et al.*, 1978; Green, 1981; Kraft *et al.*, 1986; Beckman, 1987; Jiménez-Diaz *et al.*, 1989a; Fig. 3).

Westerlund *et al.* (1974), in experiments under controlled conditions observed that the first symptom was yellowing of lower leaves of young plants which progressed uniformly upwards. He also observed that yellowing occurred on only one side of the plant i.e. only some of the main branches showed symptoms when older chickpea plants were inoculated. This has been termed "partial wilting" (Nene *et al.*, 1978). FOC is generally thought to be a vascular parasite and the fungus can usually be isolated from organs of the plant that contain this tissue. In Spain, Trapero-Casas *et al.* (1985b) have isolated a vascular FOC which induces foliar yellowing and necrosis of the cortical collar and root. In some chickpea lines and under certain circumstances, infected plants can still produce pods but these are either empty or have small, poor quality seed (Haware, 1990).

The fungus is primarily pathogenic to chickpea, but can also invade several other crops including lentil, pea, pigeonpea, alfalfa and broad bean without causing overt symptoms (Haware and Nene, 1982a; Trapero-Casas and Jiménez-Diaz, 1985b; Cabrera de la Colina *et al.*, 1987).

1.4.4. Disease development and symptom expression

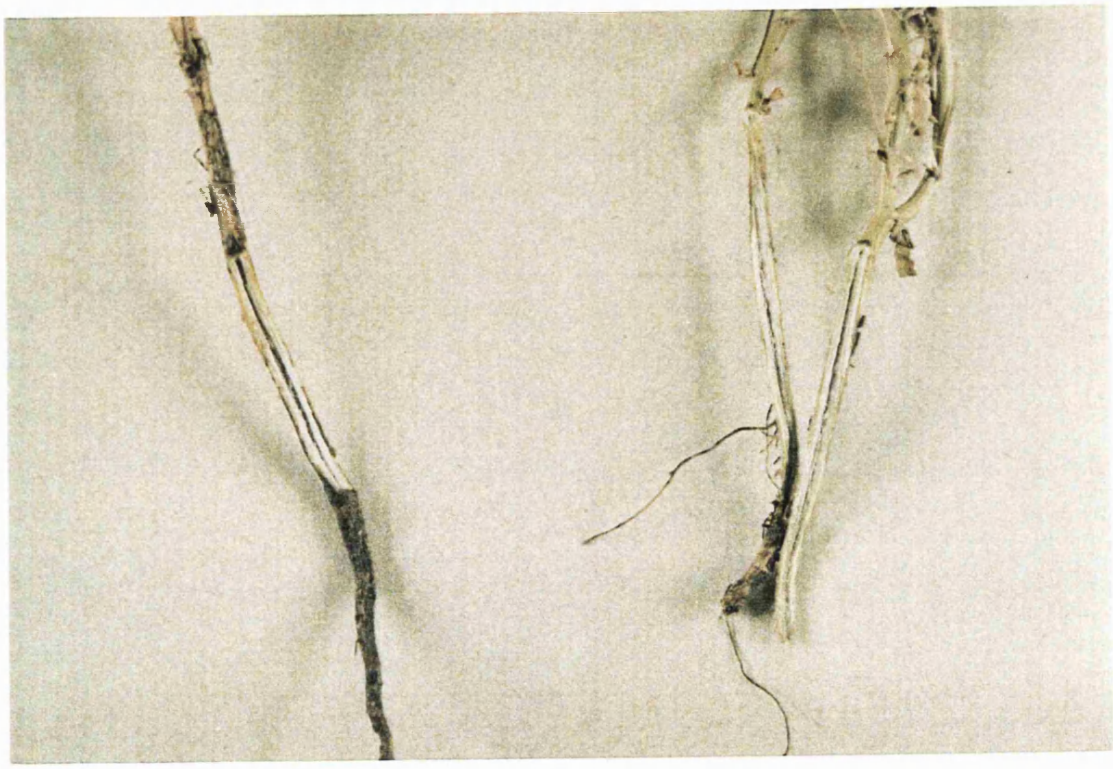
Several workers have observed different patterns in the development of wilting symptoms when chickpea is exposed to the pathogen (Sattar, 1953; Erwin, 1957; Raheja

Fig. 3:

Vascular discoloration of the stem due to the internal blackening of the xylem vessels caused by *Fusarium* wilt.

Upper - General view of the infected plant.

Lower - Close up of the stem cut longitudinally above the collar region.



and Das 1957; Erwin and Snyder, 1958). All of them reported two distinct wilting reactions, namely "early" and "late" wilting.

Chauhan (1962b) examined the development of wilt symptoms during the growth cycle of chickpea more critically and proposed four types:

1. Wilting of the whole plant at an early stage (<30 days old).
2. Wilting of the whole plant after flowering.
3. Withering of part of the plant before flowering.
4. Withering of part of the plant after flowering.

Much later, the late wilting reaction was again observed by several other workers (Nema and Khare, 1973; Saraf, 1974; Sinha, 1973; Chandra *et al.*, 1974) and the genetics of this character were studied (Upadhyaya *et al.*, 1983a, b; Singh *et al.*, 1987a, b; Van Rheenen *et al.*, 1989).

The development of FOC has not been intensively studied. Such studies would improve our understanding of the dynamics of the disease and might provide information useful for control measures (Nelson *et al.*, 1989). Campbell *et al.*(1980, 1984) and Campbell and Powell (1980) have developed disease progress models for soilborne pathogens and these have been critically reviewed by Pfender (1982) and Gilligan (1983, 1990). The only attempt to use such models for FOC was made by Jiménez-Diaz and Trapero-Casas (1985). In a study of fungicide treatments and host resistance to control wilt and the root rot complex of

chickpeas they used the "simple interest disease" (SID) model (sometimes known as the monomolecular model) and "compound interest disease" (CID) model (sometimes known as the logistic model) as defined by Van der Plank (1963). The CID model fitted their data better than the SID model but they did not relate this to the polycyclic nature of the disease. Later, Jiménez-Diaz *et al.* (1990b) used the area under the disease progress curve (AUDPC) to study the effects of planting date on development of *Fusarium* wilt in different chickpea cultivars.

Many studies of the effects of environmental factors on root diseases have relied on single measurements of disease toward the end of the growing season (Verma *et al.*, 1974). By their nature these do not consider disease progression with time, which is important in understanding the interaction of plant, pathogen, and environment.

1.4.5. Ecology and epidemiology

FOC is soilborne and seedborne. It can survive in soil in the absence of the host for more than 6 years (Haware *et al.*, 1986) and chlamydospores have been observed in the hilum region of infected seed. Seedborne inocula can be eradicated by dressing with Benlate T (30% benomyl + 30% thiram) at the rate of 0.15% (Haware *et al.*, 1978, 1986).

The precise mode of entry of FOC into the host is not clear and with the exception of the work of Jiménez-Diaz *et al.* (1989a), there are hardly any reports in the literature on the subject. Jiménez-Diaz *et al.* (1989a)

indicated that infection occurs mainly through the cotyledon and adjacent tissue or by the main root but not at the root apex. Penetration of the root surface, according to Van Rheenen *et al.* (1989), is occasionally prevented by physical and chemical barriers.

Schroth and Hildebrand (1964) suggested that root exudates may inhibit or stimulate the process of pathogenesis in diseases caused by soilborne pathogens, including vascular wilt pathogens. In support of this hypothesis, Haware and Nene (1984) found that the resistant chickpea cultivar CPS-1 produced a root exudate that inhibited spore germination and delayed mycelial growth, while the root of JG-62, a susceptible cultivar, produced an exudate that stimulated spore germination.

Disease development and symptom expression in wilt of chickpea caused by FOC are influenced by variety, environmental and biotic factors which include soil and air temperature, soil moisture, soil nutrients, soil bulk density, inoculum density, plant density and plant age (Jiménez-Diaz *et al.*, 1990c). The limited data available on the role of these factors are reviewed in the following paragraphs.

1.4.5.1. Soil and air temperature

Soil and air temperature are key factors in determining disease development and the expression of wilt symptoms. The optimal air temperature for growth of the fungus *in vitro* was between 20°C and 25°C (Van Rheenen *et*

al., 1989) but Bhatti and Kraft (1992a), in a paper published while the work for this thesis was in progress, reported that, in temperature controlled conditions, wilt was severe at 25°C and 30°C, moderate at 15°C and 20°C and absent at 10°C even at high inoculum levels of the fungus.

For soil temperature, the only report is that of Chauhan (1963a). He observed that disease incidence was maximum at a soil temperature of 25°C but was considerably reduced at higher or lower temperatures. At 15°C there was no disease development.

1.4.5.2. Inoculum density (ID)

Knowledge of inoculum density is fundamental for any interpretation of varietal reaction to FOC and to soilborne pathogens in general. When this thesis was begun, there were practically no published reports on the effect of inoculum density of FOC on *Fusarium* wilt development in chickpea. Van Rheenen *et al.* (1989) reported from unpublished data that an inoculum level of 67 to 483 propagules/g of soil caused 100% mortality in susceptible cultivars at ICRISAT and, very recently, Bhatti and Kraft (1992a) found that an ID of 500 colony-forming units per gram of soil (cfu/g) was enough for FOC to be highly destructive, providing the temperature was between 25 and 30°C. At 15°C and 20°C an ID of 500 and 1000 cfu/g of soil of FOC gave only moderate disease symptoms, while at 10°C ID levels as high as 5000 cfu/g of soil did not induce any wilt symptoms (Bhatti and Kraft, 1992a).

1.4.5.3. Soil bulk density and soil moisture

Bhatti *et al.* (1992b) found that wilt severity was the same in soils of bulk density 1.2 g/cm^3 (loose soil) and 1.5 g/cm^3 (compact soil).

High soil moisture levels have been reported to be conducive to disease. For example, Chauhan (1963b) found that wilt severity was greater at high soil moisture levels (25% soil dry weight) compared with lower moisture levels, while Bhatti and Kraft (1989) found that both wilt and the rhizosphere population of FOC increased with increasing soil moisture levels in the range 12 to 25%.

1.5. RACES OF *FUSARIUM OXYSPORUM* f.sp. *CICERI* (FOC)

FOC shows clear pathogenic variability and the existence of distinct physiologic races is now well established in all areas where the fungus is present except North Africa and Mexico.

Haware and Nene (1982b) were the first to show the occurrence of races and identified four among Indian isolates which they designated 1,2,3,4 on the basis of the differential reactions of 10 chickpea lines. Cabrera de La Colina *et al.* (1985) working with FOC isolates collected in southern Spain, identified two more races and named them 0 and 5. Later Phillips (1988) reported a race which he designated as 6, on the basis of the disease reaction of the 10 differentials of Haware and Nene (1982b) in naturally infested field at San Luis Obispo, California.

Jiménez-Diaz *et al.* (1989b), continuing the work of

Cabrera de La Colina *et al.* (1985), identified one more Spanish race which was also designated as race 6. In this work, the differential set used by Haware and Nene (1982b) was extended by five cultivars, 12-071/10054, ICCV 2, ICCV 4, PV-24 and P-2245. This allowed the differentiation of race 6 from race 1 as cv. 12-071/10054 is susceptible to race 1 but resistant to race 6. They also confirmed that race 0 was not pathogenic to JG-62 (which was susceptible to all other races) and was the least virulent but most prevalent race in southern Spain.

Buddenhagen and Workneh (1988) stated that at least two pathogenicity groups of FOC existed in California. Isolates of these groups were compared with the Spanish races and it was concluded that they resembled races 0, 1, 5 and 6 (Kraft *et al.* 1994).

The Spanish races were divided into two groups. The first group, which is a vascular, yellowing pathotype, contains race 0 and the second, which is considered to be a vascular wilt pathotype, contains races 5 and 6 (Jiménez-Diaz *et al.*, 1989b; Jiménez-Diaz *et al.*, 1989c). Race 0 besides being widespread in southern Spain also occurs in Italy (Frisullo *et al.*, 1989). It may, furthermore, be present in Tunisia but this supposition is based soley on the resistance of cv. JG-62 in the wilt-sick plot at Béja (Nene and Sheila, 1986; Halila, personal observations). Clearly, there is a need to conduct detailed research work in Tunisia in order to clarify the race status in the country.

1.6. RESISTANCE TO FOC

1.6.1. Sources of resistance to FOC

Resistant cultivars would be the best method for controlling FOC and extensive screening programs for wilt resistance are underway at several institutes. At ICRISAT, Hyderabad, India, field screening as well as laboratory and greenhouse screening have been developed and standardised (Nene *et al.*, 1981). In Tunisia, a wilt-sick plot has been developed at INRAT-Béja station for screening purposes (Halila *et al.*, 1984) and Singh and Reddy (1991) have reported the establishment of similar "sick plots" in many other countries including Ethiopia, Mexico, Spain (Santaella and Cordoba) and the USA. Advantages and difficulties associated with these wilt-sick plots have been discussed by Jiménez-Diaz *et al.* (1990c).

Pot screening and water-culture screening techniques under controlled environmental conditions have also been developed which allow comparison with field experiments (Nene and Haware, 1980; Nene *et al.*, 1981). Massive screening programmes of chickpea germplasm accessions maintained at ICRISAT and ICARDA were and are being executed at different locations. At ICRISAT, over 13,500 accessions from 40 countries were evaluated (Haware and Nenè, 1980b; Nene and Haware, 1980; Haware *et al.*, 1981, 1992). Among them, 150 "desi" but only 10 "kabuli" accessions were resistant to race 1. A few lines with broad-based and stable resistance to wilt and root-rots have been identified (Nene *et al.*, 1989; Van Rheenen *et*

al., 1989). These are ICC 2862, ICC 9023, ICC 9032, ICC 10803, ICC 11550 and ICC 11551.

In Spain during 1987 and 1989, 713 FLIP breeding lines from the ICARDA programme and 991 ILC lines from the ICARDA germplasm collection were evaluated for resistance in a field plot heavily infested with FOC. Nine "kabuli" types were found to be resistant but their reactions were variable when they were inoculated separately with the Spanish races 0 and 5 (Jiménez-Diaz *et al.*, 1991).

In Tunisia, systematic screening of "kabuli" chickpea accessions, maintained at the ICARDA world collection for *Fusarium* wilt resistance, was started in 1989. Part of this work is presented in this thesis.

1.6.2. Development of resistant breeding lines

The emphasis in breeding for resistance to *Fusarium* wilt has been placed mainly on "desi" types of chickpea. These efforts, which were started in the 1920s, have been concentrated on the Indian subcontinent and reviewed by Singh (1987) and Singh and Reddy (1991). For "kabuli types", breeding work started in Mexico (Singh, 1987) where high-yielding, wilt resistant cultivars, including Surutato 77, Sonora 80, and Santo Domingo have been released. The first two of these cultivars were developed by transferring genes for resistance from "desi" lines L 41 and L 1186 to "kabuli" lines Macarena and Breve Blanco (Singh, 1987). Screening was done in an infested field at Culiancan but the races present were not

documented.

Recently, wilt resistant cultivars Gavilan, Kino and Tubutama were bred at Sonora (Moralez, 1986). Similarly, two resistant "kabuli" lines, UC 15 and UC 27 were released in the USA (Buddenhagen and Workneh, 1988). In Tunisia, Amdoun 1, a large seeded, "kabuli" cultivar which was wilt resistant was obtained by single plant selection in the local landrace Amdoun (Halila *et al.*, 1984). Also, short season, high-yielding cultivars of the "kabuli" type, ICCV 2, 3, 4, and 5, have been developed at ICRISAT (Kumar *et al.*, 1985) which are resistant to race 1.

Efforts are now being made to breed "kabuli" cultivars with combined resistance to *Fusarium* wilt and *Ascochyta* blight (Halila and Harrabi, 1990).

1.6.3. Mechanisms of resistance to FOC

The mechanisms of resistance to FOC are not understood but are likely to depend on more than one process. Apart from mechanical resistance, coordinated chemical defence mechanisms, with different metabolic sites of action at different stages in the host-parasite interaction, are thought to be involved (Van Rheenen *et al.*, 1989). For example, exudates from susceptible cultivars stimulated mycelial growth and germination of conidia, while exudates from resistant cultivars inhibited these processes (Satyaprasad and Ramarao, 1983; Haware and Nene, 1984). In other work, some genotypes that are considered to be resistant became infected and showed

vascular discolouration but were not killed by the fungus (Singh and Reddy, 1991).

1.7. GENETICS OF RESISTANCE TO FOC

Knowledge of the genetics of resistance helps breeders to develop appropriate breeding strategies for resistance and the reduction of yield losses caused by disease. The results of studies of the genetics of resistance to *Fusarium* wilt are summarised in Table 1.

Ayyar and Iyer (1936) were the first to conduct research on the mode of inheritance of resistance to wilt. Their research was done under field conditions and they concluded that resistance is monogenic in nature with incomplete dominance. This finding was later confirmed by several other field studies (Pathak *et al.*, 1975; Haware *et al.*, 1980c; Tiwari *et al.*, 1981; Phillips, 1983; Sindhu *et al.*, 1983). In contrast, Lopez Garcia (1974) in another field study, reported that two pairs of recessive alleles were involved in resistance to *Fusarium* wilt.

Following the discovery of races of FOC in India, systematic work on the inheritance of resistance to race 1 was initiated under controlled conditions at ICRISAT. This quickly showed that resistance to FOC was more complex than suggested by the field studies. Kumar and Haware (1982) found that resistance to race 1 in lines CPS-1 and WP-315 was controlled by a single recessive allele at the same locus in both lines but that other genes or polygenic complexes might be involved since some crosses did not give

Table 1

Summary of the studies conducted on the genetics of resistance to *Fusarium* wilt of chickpea

Authors	Nature of inheritance	Genotype
Ayyar *et al* (1936)	Single with incomplete dominance	Strain N°468
Lopez Garcia (1974)	Two pairs of recessive genes	19 Lines
Pathak *et al* (1975)	Single recessive gene	Strain 315
Haware *et al* (1980c)	Single recessive gene	9 Lines
Tiware *et al* (1981)	Single recessive gene	WR-315
Kumar *et al* (1982)	Single recessive gene	WR-315,CPS-1
Sindhu *et al* (1983)	Single recessive gene	1231,32-35-8/7
Phillips (1983)	Monogenic recessive	7 desi lines
Smithson *et al* (1983)	Three recessive independent loci	P-436-2,CPS-1 WR-315,BG-212
Upadhyaya *et al* (1983a, b)	Two recessive genes	JG-62,C-104, H-208,K-850
Singh *et al* (1987b)	K-850 and C-104 independant recessive allele	K-850, C-104

a good fit to the expected ratio of 3 (susceptible) to 1 (resistant).

Upadhyaya *et al.* (1983a), following the work of Kumar and Haware (1982), examined the wilting reaction of the susceptible parents more critically. They observed a difference in the number of days taken to wilting by the two susceptible cultivars JG-62 (early wilter) and C-104 (late wilter). This difference was governed by a single gene with early wilting partially dominant to late wilting. They concluded that at least two genes control resistance to race 1. Continuing these studies, Upadhyaya *et al.* (1983b) confirmed that the cultivar C-104 (late wilter) appears to be different from WR-315 and CPS-1 (both resistant to race 1) by a single locus, which resulted in delayed wilting when in the homozygous recessive form. They also suggested that, to be susceptible, JG-62 (early wilter) had to carry two genes in the homozygous dominant condition (H1H1H2H2). The late wilter C-104 is homozygous recessive at the second locus (H1H1h2h2) and the resistant parents (WR-315, CPS-1, and P-436-2) are homozygous recessive at both loci (h1h1h2h2).

Singh *et al.* (1987b) crossed two late wilting parents (C-104 and K-850) and observed a resistant segregant in the F2 generation. This observation was explained by the fact that the late wilter K-850 carried a recessive gene that is different and independent of the recessive gene of C-104. When present together, they confer resistance. A

further report (Singh *et al.*, 1987a) indicated that resistance to race 1 appears to be controlled by at least three independent loci; this was based on the observation of resistant segregants recovered from crosses involving another late wilting parent (H-208) with C-104 and K-850. The parents, their reaction to FOC and their putative genotypes in these studies are listed below (Singh *et al.*, 1987b):

JG-62	early wilter	H1H1H2H2h3h3
K-850	late wilter	h1h1H2H2h3h3
C-104	late wilter	H1H1h2h2h3h3
WR-315	resistant	h1h1h2h2h3h3
P-436-2	resistant	h1h1h2h2h3h3
JG-74	resistant	h1h1h2h2h3h3
BG-212	resistant	h1h1h2h2h3h3
CPS-1	resistant	h1h1h2h2h3h3

These results have tremendous implications in breeding for resistance to race 1 of FOC, since complete resistance may be obtained from crosses of susceptible parents such as K-850 and C-105 by complementation (Singh *et al.*, 1987a, b). The parents should, however, be late wilters and this type is common among the world germplasm collection of chickpea genotypes at ICARDA.

To understand further the inheritance of resistance to FOC, studies with other late-wilting and resistant parents as well as other races should be undertaken. Very

recently, the inheritance of resistance to race 2 has been initiated at ICRISAT (ICRISAT, 1992). Preliminary results indicated that resistance is recessive.

To end this review, it is worthwhile reiterating the optimistic statement originally made by Parlevliet and adapted by Van Rheenen *et al.* (1989) with regard to resistance of chickpea to FOC:

"The oligogenic nature of the control of *Fusarium* wilt gives no reason to be alarmed. ICRISAT reports and personal communications confirm that over a period of more than 10 years and in different environments, the resistance has been durable. No case of genetic defeat of genes by pathotypes that have acquired adjusted pathogenicity or virulence has been reported and the *Fusarium* wilt case resembles those of many other diseases where simply inherited resistance has been durable (Parlevliet, 1983)."

1.8. PATHOGENIC FACTORS

1.8.1. Toxins

Many workers have investigated pathogen-produced toxins and their possible involvement in disease development. Gäumann (1954) stated that "Micro-organisms are pathogenic only if they are toxigenic: in other words, the agents responsible for diseases can damage their hosts only if they form toxins/microbial poisons that penetrate into host tissue". In this context Gäumann was using the term toxin to cover all substances produced by the pathogen which injure plants, including enzymes.

Scheffer (1983) defined toxin as a product causing obvious damage to plant tissues and having a clear role in disease development. It is seldom easy to establish this role because toxins are generally difficult to isolate from diseased plant material and because artifacts may be introduced when purified toxins are administered to the plant.

There are many species of fungal and bacterial pathogens which produce low molecular weight compounds that are toxic to plants. In some instances they exert their deleterious effects by changing the properties of cellular membranes in susceptible, but not resistant plants. The consequent release of nutrients facilitates the nutrition of the pathogen and its colonization of the plant. Characteristics of these toxins have been reviewed by Scheffer (1983).

Toxins are often classified as host-selective and host-non-selective (Strange, 1993). Host-selective toxins are normally essential for pathogenicity whereas non-selective toxins are not essential for pathogenicity but may contribute to the virulence of the parasite (Strange, 1993).

1.8.2. Enzymes

The ability of pathogens to degrade plant cells is an important requirement for virulence. Many pathogenic bacteria and fungi produce polysaccharide degrading enzymes which are active against the carbohydrates found in the

cell walls of higher plants. Pectic enzymes, for example, may reduce cell wall polygalacturonides to gels causing the occlusion of water conducting vessels (Pegg, 1981), a point of potential significance in wilt of chickpea caused by FOC.

1.8.3. Toxins and enzymes of *Fusarium* sp.

Many species of *Fusarium* produce a number of metabolites that cause mycotoxicoses in animals and humans on ingestion of infected grain and phytotoxicoses of plants (Vesonder and Hesseltine, 1981). Pegg (1981) reviewed extensively the toxins and enzymes produced by fungi causing wilt diseases. *Fusarium* species produce mostly low molecular weight toxins such as lycomarasmin, fusaric acid and fusicoccin (Pegg, 1981). The latter is also considered to be a growth regulator. In addition to these compounds, other toxins are produced by some formae speciales of *Fusarium oxysporum*. For instance, *Fusarium oxysporum* f. sp. *niveum* produces phytonivein which induced irreversible wilting in watermelon shoots (Pegg, 1981) and recently Sutherland and Pegg (1992) reported a protein from *F. oxysporum* f. sp. *lycopersici* race 1 that was toxic to protoplasts of susceptible but not resistant genotypes of tomato. However, only Chauhan (1960) has reported that the culture filtrates of FOC were toxic.

The importance of pectic enzymes in the pathogenesis of several wilts caused by species of *Fusarium* and *Verticillium* has been stressed frequently by several

authors (Cooper and Wood, 1980; Pegg, 1981). *Fusarium oxysporum* f. sp. *lycopersici* produced pectin esterases (PE), polygalacturonases (PG) and pectate lyase (PL). Their possible role in vascular browning and the wilting process was discussed by Pegg (1981). Pérez-Artes and Tena (1989) have studied the production of pectic enzymes by the Spanish races 0 and 5 of FOC and the influence on pectic enzyme production and activity of cell walls from chickpea cultivars PV-24, susceptible to both races 0 and 5, JG-62, susceptible only to race 5 and resistant cultivar WR-315. They found that both races produced PG and PL. The amount produced by race 0 was about twice the amount produced by race 5. Cell walls of the susceptible cultivars, PV-24 and JG-62, allowed similar high rates of pectic enzyme synthesis by both races whereas production of PG and PL was significantly lower when the fungus was grown on the cell walls of the resistant cultivar, WR-315. PG and PL were both able to degrade chickpea cell walls.

1.9. CONTROL OF *FUSARIUM* WILT OF CHICKPEA

FOC may be eliminated from seed using the fungicide Benlate T (30% benomyl + 30% thiram) at the rate of 0.15% (Haware *et al.*, 1978; Haware *et al.*, 1986a). Use of other fungicides, protectant or systemic, in seed dressings improved seedling emergence in moderately susceptible chickpea cultivars and delayed development of epidemics but did not provide satisfactory control of the disease (Jiménez-Diaz and Trapero-Casas, 1985).

FOC can survive in the soil for up to 6 years (Haware *et al.*, 1986b) and also in symptomless carriers (Haware and Nene, 1982a; Trapero-Casas and Jiménez-Diaz, 1985b). Therefore it is not possible to control the disease by normal crop rotation. Planting chickpea in heavily infested soil is not recommended (Haware *et al.*, 1990). Soil solarization reduced the pathogen population and the incidence of wilt with the result that plant growth and yield was improved (Chauhan *et al.*, 1988). However, cost considerations would limit the use of the technique for the control of wilt in the commercial farming of chickpea.

Date of sowing seems to have an effect on the incidence of wilt. In India, for example, it has been reported that delayed sowing usually lowers fungal attack but also yield (Padwick and Bhagwagar, 1947; Raheja and Das, 1957; Singh and Singh, 1984) while in Spain, incidence and severity of wilt disease was reduced significantly in moderately susceptible cultivars, but not in susceptible ones, when planting was advanced from spring to winter (Jiménez-Diaz *et al.*, 1986; Trapero-Casas and Jiménez-Diaz, 1990b).

Deep tillage and subsoiling decreased the incidence of disease (Dahiya *et al.*, 1988) but intercropping, plant density and fertilizer application had no effect (Zote *et al.*, 1986).

Biological control of FOC was obtained through the antagonistic activity of *Penicillium pinophilum* (Haware *et al.*, 1990) and Mukherjee (1991) found that *Gliocladium*

virens also suppressed FOC. The suppression appeared to act through competition. Suppressiveness of soil has long been appreciated as a means of controlling soilborne diseases and has been extensively studied (Baker and Cook, 1974; Albouvette *et al.*, 1980, 1982, 1985; Huber *et al.*, 1982). Baker and Cook (1974) have defined suppressive soils as those in which disease development is suppressed although the pathogen is present. Two mechanisms by which *Fusarium* wilts may be suppressed are through the inhibition of chlamydospore germination and of germ tube extension (Cook and Baker, 1983).

Although the above measures give some control, the only effective way to limit the effect of the disease when chickpea is planted in infested soil is to use resistant cultivars.

1.10. AIMS OF THE PROJECT

As already discussed, *Fusarium* wilt of chickpea is an important constraint to yield improvement of this crop in many parts of the world including Tunisia and other countries of the North African region. In order to combat the disease in these regions further work is required and this is discussed in the following paragraphs.

Studies on the disease are well advanced in India and Spain but are still limited elsewhere. Although the importance and incidence of *Fusarium* wilt of chickpea is well assessed in Tunisia (Halila, personal observations), nothing is known of the variability of the fungus.

Resistance to *Fusarium* wilt of chickpea has been identified mostly in the "desi" types (Haware and Nene, 1980b, and Haware *et al.*, 1981, 1992) rather than the "kabuli" types that are grown in the Mediterranean region. There is therefore a need to identify additional sources of resistance in "kabuli" types in order to increase the genetic variability required in chickpea breeding programs. The important findings of the inheritance of resistance to *Fusarium* wilt (see section 1.7) should encourage breeders and pathologists to keep late-wilting cultivars although they are susceptible.

The interaction of a pathogen with its host is favoured, modified or inhibited over time depending on the environment and the time over which the interaction takes place (Waggoner *et al.*, 1980; Coakley, 1989). For FOC, this interaction is not adequately documented nor understood. Under field conditions, the relation between the host, environment and the fungus may be influenced by many factors of which temperature and inoculum density are probably the most important (Bhatti and Kraft, 1992a).

With the new and strong orientation towards improving and protecting our environment, the use of chemicals will have to be decreased. Integrated control or management of diseases is gaining more attention from plant pathologists. Integrated disease management has been defined as:

"A procedure using all methods economically, ecologically and toxicologically acceptable, to keep harmful organisms below the economical threshold level, with special emphasis

on natural limiting factors" (Weltzien, 1989).

Toxins have been described in a number of well documented reports as integral factors in disease development (Scheffer, 1983 and Yoder, 1980). They have proved to be useful tools in the selection of resistant/tolerant plants (Earle, 1978; Daub, 1986; Alam, 1989) since insensitivity to toxins can, theoretically at least, be used as the basis for selecting germplasm with resistance to the pathogen (Gengenbach *et al.*, 1977). It was considered that, at least in the initial stages of screening, bioassays of culture filtrates of FOC using host cells might provide a means of rapidly detecting susceptible genetic material.

In an attempt to contribute to the understanding of the above factors, this project has the following aims:

1. To identify the organisms that cause wilt in the chickpea growing area of Tunisia and study their morphological and pathogenic variability.

2. To screen part of the ICARDA world germplasm chickpea collection to the organisms identified in the previous paragraph.

3. To contribute to the understanding of the progress of disease by examining the relationships between available environmental data and the progress of wilt under northern Tunisian conditions.

4. To investigate the effect of temperature and inoculum density on disease severity under controlled conditions.

5. To test culture filtrates of the organisms identified in paragraph 1 for toxicity and assess the possibility of their use in screening for resistance.

CHAPTER 2

CULTURAL VARIATION AND PATHOGENIC VARIABILITY OF *FUSARIUM OXYSPORUM* F.SP. *CICERI*

2.1. INTRODUCTION

Wilt caused by *Fusarium oxysporum* Schlecht. emend. Snyd. and Hans. f.sp. *ciceri* (Padwick) Snyd. and Hans., hereafter designated FOC, is probably the most widespread disease of chickpea and has been reported from 23 countries around the world (Nene *et al.*, 1989). Regular surveys since 1985 have revealed that a disease corresponding in symptoms to wilt caused by FOC is a major problem in Tunisia where losses can be total (Halila *et al.*, 1984; Halila and Harrabi, 1990). The causal agent is estimated to be present in 30-40% of the fields in Tunisia (Halila, unpublished data) but no detailed studies have been made of its morphological and pathogenic variability.

2.2. MATERIALS AND METHODS

2.2.1. Acquisition of diseased plants

Infected plants were collected during the course of a chickpea disease survey conducted in the spring of 1987 in the main chickpea growing areas of northern Tunisia. Five plants with symptoms typical of wilt, i.e. drooping leaves with or without chlorosis, were randomly chosen from 39 chickpea fields in the Mateur region and the

wilt sick plot of the INRAT/Béja station (Fig. 4). In total 251 samples were collected.

2.2.2. Isolation and characterisation of fungi

The tops and lower roots of plants were removed leaving a region extending 5 cm above and below the crown. After washing in running tap water to remove adhering soil, the samples were cut into 5 or 10 mm-long sections and surface disinfected by dipping for 1 to 2 min in 1% sodium hypochlorite. Two pieces from each plant were split lengthwise and checked for vascular discoloration and four to six pieces from each plant were plated on potato-dextrose agar (PDA). The plates were incubated on benches in a growth chamber at 25°C for 8 days with a 12 h photoperiod of fluorescent and near ultraviolet light (NUV) at 10 $\mu E\ m^{-2}\ sec^{-1}$. When fungi began to grow from host tissue, mass transfers of mycelium from normal, healthy colonies were placed in culture tubes. From these, single-spore cultures were established and transferred to freshly made PDA.

Morphological characteristics were studied on 7 day old cultures. These included colour and type of mycelium, colour of substratum, presence and dimension of micro- and macroconidia and zonation. Mean radial growth rates per day were calculated from colony diameters measured 2 and 7 days after inoculation. Representative cultures were preserved in tubes containing 10 g of sterilized, fine riverbed sand at 4±1°C (Haware and Nene, 1982b).

Fig. 4:

A farmer's field in northern Tunisia (Mateur area) showing a chickpea crop affected by *Fusarium* wilt. Initial symptoms occur in patches (**upper picture**), subsequently the whole crop dies and dries out (**lower picture**).

2.2.3. Acquisition of fungal races

Races 1, 2, 3, and 4 were kindly supplied by M.P. Haware, Legume Program, ICRISAT, Patancheru, India and races 0, 5 and 6 were a gift from R.M. Jiménez-Diaz, Departamento de Agronomia, Escuele Técnica Superior de Ingenioros Agronomos (ETSIA), Universidad de Cordoba, Spain. All strains were received as slants on PDA in tightly-closed glass tubes. After multiplication by subculturing on PDA medium, they were stored as described above.

2.2.4. Acquisition and characteristics of seed

Two cultivars from the ICARDA germplasm collection were used in initial pathogenicity studies of the Tunisian isolates of FOC. These were ILC 482, a "kabuli" type with a 100-seed weight of about 28 g, and ILC 223 a "near-kabuli" type with a slight maroon colour and smaller 100-seed weight (about 20 g). Both lines appeared susceptible to *Fusarium* wilt in the field but symptoms were more severe in ILC 223 (Halila, unpublished data).

FOC isolates were tested with 16 differential varieties. These consisted of the 10 lines used by Haware and Nene (1982b), the 5 lines used by Jiménez-Diaz *et al.* (1989b) and one line from ICARDA. The differentials and their origins are given in appendix 1.

Seed of the ICRISAT lines were obtained from M.P. Haware and seed of line 12-071/10054 as well as the "kabuli" cultivars PV-24 and P-2245 were kindly provided by A.

Trapero-Casas, Universidad de Cordoba, Spain.

The seed of the differential varieties was multiplied in an insect-proof cage on wilt free soil at INRAT/Bousalem station in northern Tunisia. Plants were protected with fungicide sprays against *Ascochyta* blight and any that appeared abnormal were discarded. Healthy seed was harvested and stored at 4°C.

2.2.5. Inoculation methods

Two methods were used to test the pathogenicity of FOC isolates: 1. The spore suspension technique used by Wensley and Mckeen (1962) to study vascular wilt in melon and 2. the pot-inoculation method developed by Haware and Nene (1982b). For both methods inoculum was prepared from infested sand by dispersing a small amount on PDA plates and incubating for 7 days at 23-25°C under the light régime described in section 2.2.2.

For the first method, conidial suspensions were prepared by flooding the cultures with sterile water and filtering through three layers of cheese-cloth. The suspension was adjusted, using a haemocytometer (Fuchs and Rosenthal model), to a concentration of 10^6 conidia/ml and dispensed in 20 ml glass tubes (15 ml/tube).

For the second method, an agar disk (4 mm diam.) cut from the edge of a 7-day-old FOC colony growing on PDA, was transferred to a 250 ml conical flasks containing 100 g of 9:1 sand-maize medium (Haware and Nene,1989b). Flasks were incubated for two weeks at 25-27°C. Stocks of soil

(vertisol) and riverbed sand mixture (1:1) were autoclaved twice at 110°C for 1.5 h in batches of 20 Kg, inoculated with the contents of the flasks and put in 15 cm plastic pots. Inoculation was done by mixing the soil and the contents of the flasks (20:1 w/w) in a heavy duty cement mixer.

Chickpea seed was surface-disinfected by immersion in a 2.5% solution of sodium hypochlorite for 2-3 min, rinsed twice with sterile distilled water and air-dried. Plants were grown for 7-8 days in pans of autoclaved sand placed on a greenhouse bench.

For the first inoculation method, the seedlings were washed free of sand and transferred, without intentional wounding, to the tubes containing the conidial suspension. Plants were held in position with sterile cotton-wool plugs. Three plants of each variety were used for each isolate and replicated six times. Control plants were placed in tubes containing washings from uninoculated PDA plates. Tubes were randomly distributed in a perforated tray on a gyratory shaker (Model G10, New Brunswick Scientific Co., Edison, New Jersey, USA) at 90-100 rpm (2.5 cm. diam gyrations) and left on a bench of a growth chamber where the temperature was maintained at 27±1°C during the day and 21±1°C at night. The light was from warm white fluorescent tubes, giving a 14h photoperiod and about 70 $\mu E\ m^{-2}\ sec^{-1}$. Sterile distilled water was added every two days to make up for water loss. The experiment was repeated twice.

For the second inoculation method six plants (7-days-old) were transplanted to each pot of infested soil mixture. Control plants were grown in the uninoculated soil mixture. Pots were arranged in a randomized complete block design with three replicates for each isolate-variety combination on the bench of the growth chamber with the same conditions as just described. Pots were watered as required and 100 ml of Hoagland nutrient solution (Hoagland and Arnon, 1950) was added weekly.

Before use, pans and pots were washed in running water, dipped for 5 minutes in 5% sodium hypochloride solution, rinsed twice in distilled water and air dried. The heavy duty mixer was disinfected similarly three times after mixing the inocula of each isolate of FOC in order to prevent cross-contamination with isolates.

2.2.6. Scoring disease symptoms

For the first inoculation technique using conidial suspensions, symptoms were scored at 5 days after the initiation of the experiment and then every one to two days for 10 days. Plants were scored on a 0 - 2 scale:

0 = No symptoms

1 = Mild wilting with yellowing of lower leaves

2 = Total collapse of the plant

For the second inoculation technique involving pot culture in soil infested with FOC, disease reaction was assessed every 7 days from 5 days after transplanting by counting the numbers of wilted plants.

For race identification, numbers of diseased plants were recorded once at 40-43 days after transplanting and scored as follows:

R: Resistant interaction with 0-20% plants wilted.

M: Moderately susceptible interaction with 21-50% plants wilted.

S: Susceptible reaction with more than 50% plants wilted.

The experiment was repeated three times for the Tunisian FOC isolates and once for the defined races.

Re-isolation of the fungus from plants showing symptoms was performed by plating stem pieces from the crown region onto PDA following the method described in section 2.2.2. Vascular discoloration was checked by splitting the stem and tap roots of three plants chosen randomly. Analysis of variance (ANOVA) was performed on the data obtained from each inoculation method.

2.3. RESULTS

2.3.1. Identification of fungi isolated from chickpea plants grown in Tunisia

Isolates from plants, showing clear vascular discoloration at the collar level, were grouped into five classes based on their morphology. Two of the classes were found in the Mateur region and the other three were from the Béja wilt sick plot. Representatives of each class were identified as FOC following the scheme of Nelson *et al.* (1983). Identification of representatives of three of the

classes, two from the Béja wilt sick plot and one from the Mateur area as FOC was confirmed by D. Brayford, International Mycological Institute, Bakenham Lane, Egham, Surrey, U.K. These are designated as follows:

Strain	Herb.I.M.I number
FG_3	328268
F/SG_3	328269
FG_4	328270

The two classes which could not be confirmed as FOC were not included in the experiments reported in this thesis but a further isolate, identified as *Fusarium solani* (Mart.) Sacc., has been included for morphological characterisation only.

In addition, single-sporing mass fungal cultures from plants grown in the Béja wilt sick plot yielded *Gliocladium* spp. in 19 samples. These were identified by M.A.J. Williams, International Mycological Institute as *Gliocladium catenulatum* J. Miller, Giddens and Foster and *Gliocladium roseum* Bainier.

2.3.2. Characterisation of *Fusarium* species isolated from chickpea plants grown in Tunisia

The macroscopic growth characters and growth rates ofthe *Fusarium* species isolated are presented in Fig. 5 and

Table 2. Aerial mycelium was moderate to abundant for FG_3 and F/SG_3 and the *F. solani* isolate, whereas for FG_4 it was scanty. The colour of the substratum for the FOC isolates was a creamy orange and in the case of FG_4 black around the point of inoculation (Fig. 5). Zonation was often present in the *F. solani* isolate and sometimes present in the FOC isolates, being most pronounced in FG_3. Of the four isolates, only FG_3 had a significantly lower growth rate (Table 2).

The morphological characteristics of the microconidia and macroconidia of the four isolates are presented in Table 3. Microconidia of all isolates were variable in length but the breadths of isolates FG_3 and FG_4 were similar and greater than those of F/SG_3 (Table 3). The breadth of microconidia of the *F. solani* isolate was also variable. Microconidia were fewest in *F. solani* but were moderately abundant to abundant in the FOC isolates.

The breadth of macroconidia varied over a similar range for all isolates but there were wide differences in length. The smallest were those of FG_4 which also varied over a smaller range and were fewer. Septation varied from 2-4 for all isolates except *F. solani* in which it varied from 2-5 (Table 3).

No chlamydospores were present at the time when morphological characters were examined (7 days after subculture).

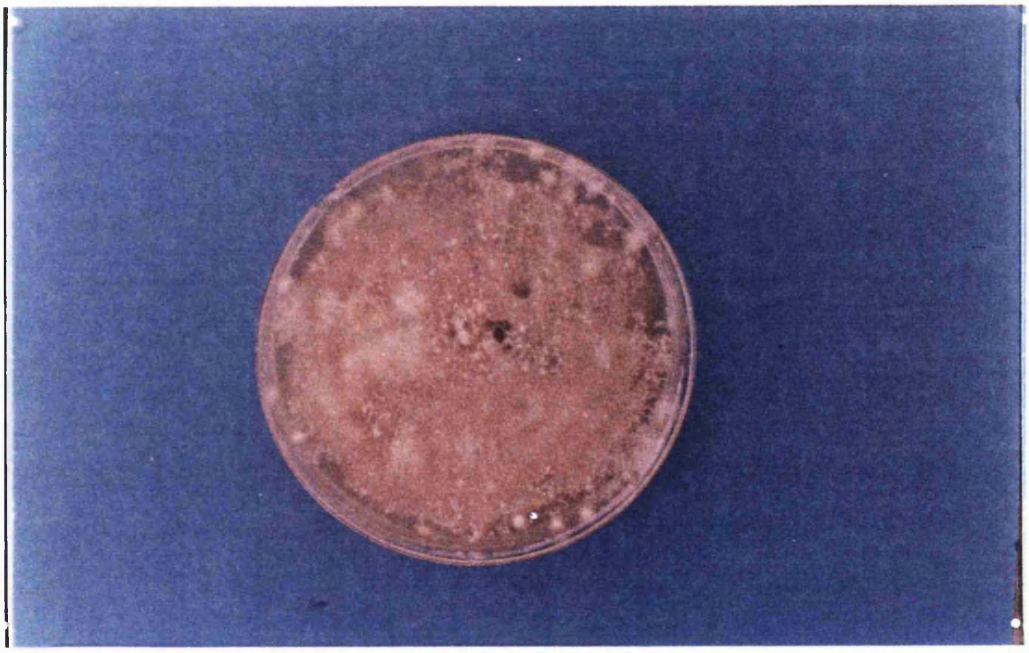
Fig. 5:

Typical sample plates of different isolates of FOC

Upper: FG_3 isolate originating from the wilt sick plot at Béja

Middle: F/SG_3 isolate originating from the wilt sick plot at Béja

Lower: FG_4 isolate originating from the Mateur area



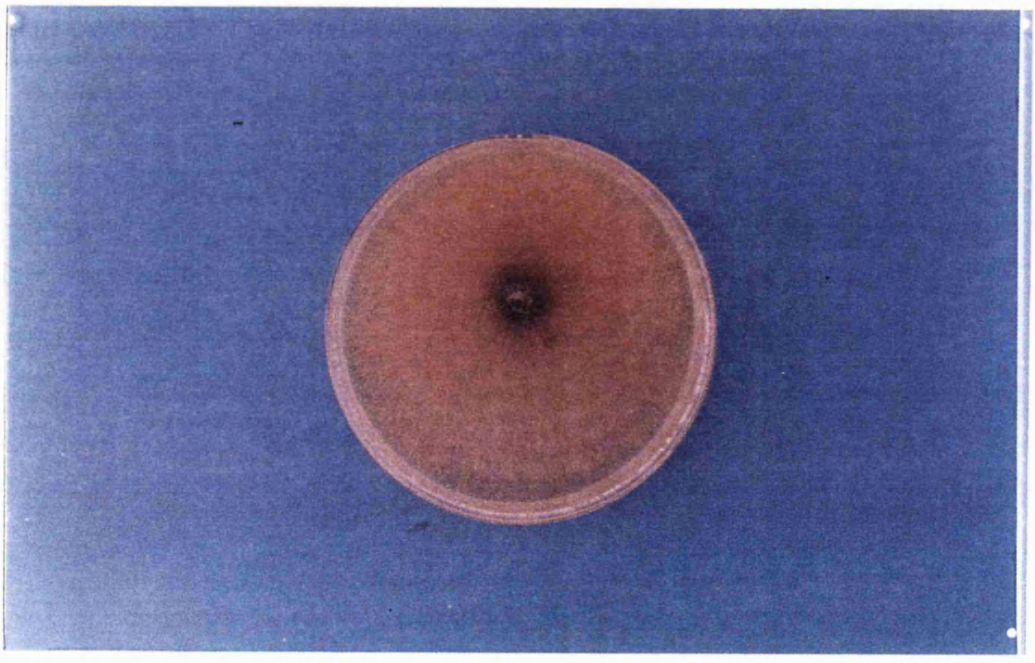


Table 2

Cultural characteristic of *Fusarium* isolates.

Fusarium isolates	Aerial mycelium	Colour of substratum	Texture	Zonation	Radial growth rate per day (mm)
FOC isolates					
FG_3	Moderate to abundant. Irregular distribution over the medium but never covers the whole plate. Sometimes collapsed or semi-submerged. White. Moderate growth.	Orange cream to light cream	Cottony	Sometimes present	3.53 ± 0.91
F/SG_3	Similar to FG_3, with more mycelium. White cream. Fast growth.	Orange cream to light cream	Cottony	Sometimes present	5.35 ± 0.39
FG_4	Scanty or absent. Semi-submerged. Dark mauve color around the point of inoculum. Fast growth.	Deep cream orange and black in the centre	-	Dark zone close to the center.	5.53 ± 0.75
Fusarium solani					
FG_1	Very Scanty. Dispersed in concentric circles around the inoculation point. White to gray white. Moderate growth.	Gray white to light cream	Cottony	Very often present	5.08 ± 0.45

Table 3

Conidial characteristics of *Fusarium* isolates.

Fusarium isolates	Microconidia			Macroconidia			
	Length (μ)	Breadth (μ)	Presence[1]	Length (μ)	Breadth (μ)	Septation	Presence
FOC isolates							
FG_3	5.63-12.88 (9.50)[2]	2.41-3.22 (3.06)	+ +	19.32-43.47 (31.88)	3.22-4.02 (3.38)	2-4	+ + +
F/SG_3	8.05-11.27 (9.02)	1.61-2.41 (2.09)	+ + +	11.88-40.25 (28.01)	3.22-4.83 (3.54)	2-4	+ + +
FG_4	6.44-11.27 (8.69)	2.41-3.22 (2.74)	+ + +	11.27-22.54 (14.81)	3.22-4.02 (3.38)	2-4	+
Fusarium solani							
FG_1	6.44-12.88 (10.46)	1.61-4.02 (3.86)	+	30.59-51.52 (27.83)	3.22-4.83 (4.51)	2-5	+ + +

1: + : Moderate
+ + : Moderately abundant
+ + + : Abundant
2: Figures in parenthesis are means.

2.3.3. Pathogenicity tests

2.3.3.1. Pathogenicity of Tunisian isolates to cultivars ILC 223 and ILC 482

Symptom expression was more rapid with the spore suspension technique than with the pot method (Tables 4 and 5). In the spore suspension technique, yellowing of lower leaves started 5 days after inoculation for all three isolates with ILC 223 and 6 days after inoculation for FG_3 and F/SG_3 with ILC 482 but not with FG4 until 10 days after inoculation. FG_3 appeared to be more aggressive than F/SG_3 and FG_4 since ILC 223 and ILC 482 wilted and collapsed 7 and 12 days after inoculation respectively with this isolate, whereas for FG_4 and F/SG_3, more than 15 days were needed for these reactions (Table 4). All plants showed a susceptible reaction at 21 days after inoculation and the pathogen was re-isolated from them. Non-inoculated seedlings remained green for more than 25 days.

In the pot screening technique there was a significant interaction ($P = 0.05$) between isolates and cultivars (Table 5). As in the spore suspension technique, symptoms developed more quickly in ILC 223 than in ILC 482.

FOC was reisolated from all plants showing symptoms whereas plants in non-inoculated pots were grown for more than 60 days with no visible wilt symptoms and no fungus could be isolated from them.

Table 4

Pathogenicity trials performed with three FOC isolates and two chickpea varieties using the spore suspension technique.

Varieties and FOC isolates	Wilt reaction[1] observed after								
	5 days	6 days	7 days	8 days	10 days	12 days	13 days	15 days	21 days
ILC 223									
FG_3	1	1	2	2	2	2	2	2	2
F/SG_3	1	1	1	1	1	1	1	1	2
F_4	1	1	1	1	1	1	1	1	2
ILC 482									
FG_3	0	1	1	1	1	2	2	2	2
F/SG_3	0	1	1	1	1	1	1	1	1
FG_4	0	0	0	0	0	1	1	1	2

(1) 0 : No symptoms, 1: Mild wilting with yellowing of lower leaves, 2: Total collapse of the plants.

Table 5

Pathogenicity tests performed with three FOC isolates and two chickpea varieties [2] using the pot screening technique.

Varieties and FOC isolates	Wilt incidence (%)[1] at (days after inoculation)						
	5 days	12 days	19 days	26 days	33 days	40 days	47 days
ILC 223							
FG_3	0	26a[3]	60d	100f	100	1001	100
F/SG_3	0	20b	51d	89g	100	1001	100
FG_4	0	22b	55d	92h	100	1001	100
ILC 482							
FG_3	0	0c	10e	75i	75	90m	100
F/SG_3	0	0c	9e	80j	80	95m	100
FG_4	0	0c	10e	87k	87	94m	100

(1) wilt incidence = $\frac{\text{number of wilted plants}}{\text{total number of plants}} \times 100$

(2) Total number of plants were 18 for each test.

(3) Figures followed by the same letters are not significantly different.

2.3.3.2. Pathogenicity of defined races and Tunisian isolates of FOC for differential cultivars

Results of pathogenicity tests showed that the Tunisian isolates had essentially the same reaction on the differential cultivars as race 0 (Table 6). In particular, unlike races other than race 0 they were not pathogenic to cultivar JG-62. However, race 0 induced a moderate reaction with cultivar PV-24 whereas this cultivar was clearly susceptible to the Tunisian isolates. The reactions of the other races (1,2,4, 5 and 6) were as previously reported (Haware and Nene, 1982b; Jiménez-Diaz *et al.*, 1989b).

2.3.3.3. Relation of cultural characteristics and pathogenicity

There was no relation between characteristics of the Tunisian isolates of FOC in culture and pathogenicity. The Tunisian isolates induced leaf yellowing and stunting followed by plant death in susceptible cultivars, with the exception of L-550 where progressive yellowing was observed with time. Vascular discoloration was always associated with susceptibility.

2.3.3.4. Pathogenicity of *Gliocladium* species

Both *Gliocladium* species were pathogenic to chickpea seedlings in the spore suspension method (data not shown) but were not with the pot technique.

Table 6
Reactions[1] of 16 differential chickpea varieties to defined FOC races and to FOC isolates collected in Tunisia

Varieties	Race 1	Race 2	Race 3	Race 4	Race 0	Race 5	Race 6 Spain	FG_3 [2]	F/SG_3	FG_4
JG-62	S	S	S	S	R	S	S	R-R-R	R-R-R	R-R-R
C-104	M	S	S	S	M	S	M	S-M-M	M-M-M	S-M-R
JG-74	R	M	R	R	R	M	R	R-R-R	R-R-R	R-R-R
CPS-1	R	S	S	M	R	M	R	R-R-R	R-S-R	R-R-R
BG-212	R	S	M	M	R	R	R	R-R-R	R-R-R	R-R-R
WR-315	R	R	M	R	R	R	R	R-R-R	R-R-R	R-R-R
Annigeri	S	S	S	S	R	R	R	R-R-R	R-R-R	R-R-R
Chafa	S	S	M	S	R	R	R	R-R-R	R-M-R	R-R-R
L-550	S	S	M	S	S	S	S	S-S-M	S-M-S	M-M-S
850 3/27	S	M	M	S	R	R	R	R-R-R	R-R-R	R-R-R
12-071/10054	M	R	R	R	S	R	R	S-S-M	M-M-S	S-S-S
ICCV 2	R	M	M	M	R	S	M	R-R-R	R-R-M	R-R-R
ICCV 4	R	S	S	S	R	S	M	R-R-R	R-M-R	R-R-R
PV-24	S	S	S	S	M	S	S	S-S-S	S-S-S	S-S-S
P-2245	S	S	S	S	S	S	S	S-S-S	M-S-S	M-S-M
ILC 223	S	S	S	S	S	S	S	S-S-S	S-S-S	S-S-S

(1) Readings were taken 40 to 43 days after seedling transplantation (18 seedlings for each variety and isolate). Disease reaction were assessed using the scale of Haware and Nene (1982): R: Resistant (0-20% WI), M: Moderately susceptible (21-50% WI) and S: Susceptible (≥51% WI).
(2) Results for Tunisian isolates, FG_3, F/SG_3 and FG_4 were for three experiments

2.4. DISCUSSION

FOC was isolated from 246 plants showing symptoms typical of *Fusarium* wilt in the field, i.e. epinasty, uniform or non-uniform wilting of some leaves or branches and vascular discoloration extending above the soil line as well as below the collar region. The remaining 5 plants yielded *Fusarium solani* (Mart.) Sacc. as confirmed by the International Mycological Institute. These isolates were from plants which were already wilted and dry and which were collected late in the spring from a low-land field in the Mateur area. In addition, *Gliocladium catenulatum* and *G. roseum* were found associated with plants growing in the Béja wilt sick plot.

The FOC strains isolated in this study, like those in other studies (Chattopadhyay and Sen Gupta, 1967; Haware *et al.*, 1986b; Desai, 1986) varied in morphological and cultural characteristics. However, variation was not apparent in pathogenicity studies, representatives of all three morphological classes causing similar symptoms on all cultivars of plants tested and defining them as race 0, despite the significantly lower growth rate of isolate FG_3. This contrasts with results from other plant pathogenic fungi. For example, Brasier and Webber (1987) found that those isolates of *Ophiostoma ulmi*, the causal agent of Dutch Elm Disease, that were most pathogenic had the fastest growth rate.

Testing for pathogenicity by the spore suspension method, which was very similar to the water culture

technique used by Nene and Haware (1980), was rapid, reproducible and required little space. The pot screening method was also effective and is closer to natural growing conditions. However, it is more laborious and symptoms take longer to appear. Both techniques discriminated between chickpea cultivars used in pathogenicity tests, demonstrating that cultivar ILC 223 was more susceptible than cultivar ILC 482.

Fusarium oxysporum Schlecht. is known for its pathogenic specialisation. Armstrong and Armstrong (1981) listed 75 formae speciales of the fungus and more than 60 races were identified within these formae. Attempts to identify physiological races within FOC were made as early as 1962 (Chauhan, 1962e) but it was not until 1982 that Haware and Nene (1982b) were able to report unequivocally the existence of four races in India. Three more races were reported from Spain (Jiménez-Diaz *et al.*, 1989b).

In the present study the ICRISAT criteria for race identification were used (Haware and Nene, 1982b). These rely on wilt incidence in contrast with the criteria of the Spanish researchers who use a scale based on severity of symptoms (Jiménez-Diaz *et al.*, 1989b). Both criteria produced similar results and confirmed differences between the Indian and Spanish races. Furthermore, comparison of differential interactions was improved by adding new genotypes to the differential set and this has allowed the classification of Spanish pathotypes of FOC into races 0, 5 and 6 (Jiménez-Diaz *et al.*, 1990b) and the Californian

pathotypes into races 0, 1, 5 and 6 (Kraft *et al.*, 1994).

In the present study the three Tunisian isolates were assigned to race 0. This assignation was based principally on their avirulence to the "desi" cultivar JG-62, which is susceptible to all other races of the pathogen, as well as their moderate to high virulence for cultivars C-104, 12-071/10054, PV-24, P-2245 and ILC 223 (Table 6). The isolates caused vascular discoloration and typical wilting symptoms with drooping leaves which sometimes became chlorotic.

The finding that the two species of *Gliocladium* were pathogenic to chickpea when tested by the spore suspension method but not when tested by the pot technique is puzzling. One explanation could be that these species produce phytotoxic compounds which are adsorbed on soil particles. Species of *Gliocladium* are well known antagonists of a number of soil-borne plant pathogens and in some instances have been shown to exert this activity throught the production of specific compounds such as gliotoxin (Roberts and Lumsden, 1990). In the context of this thesis it is interesting that Mukherjee (1991) found *Gliocladium virens* had an antagonistic effect on FOC but did not affect the germination and growth of chickpea.

CHAPTER 3

REACTION OF CHICKPEA GERMPLASM TO *FUSARIUM* WILT UNDER FIELD CONDITIONS

3.1. INTRODUCTION

Several workers have observed different patterns in the development of wilting symptoms when chickpea is infected with *Fusarium oxysporum* f. sp. *ciceri* (Sattar, 1953; Erwin, 1957; Raheja and Das, 1957; Erwin and Snyder, 1958; Chandra *et al.*, 1974). Chauhan (1962b) examined the development of wilt symptoms during the growth cycle of chickpea and proposed four types of wilting based on the timing of the appearance of symptoms in relation to flowering (section 1.4.4). A late wilting reaction was observed by several other workers but such varieties were considered to be susceptible and therefore discarded in breeding programmes (Nema and Khare, 1973; Chandra *et al.*, 1974; Saraf, 1974). Later the genetics of the late wilting character were studied (Upadhyaya *et al.*, 1983a, b; Singh *et al.*, 1987a, b). The results showed that complete resistance could be obtained from crosses involving parents with the late wilting character (section 1.7). In order to identify genotypes responding in this way under Tunisian conditions as well as those with complete resistance, representative accessions of the ICARDA chickpea collection were screened in the wilt sick plot at Béja.

3.2. MATERIALS AND METHODS

3.2.1. Development of a wilt sick plot at Béja station (WSP)

Béja experimental station is located in northern Tunisia in the cereal and food legume belt at a latitude of 36.52^0 north and longitude 9^0 east. The altitude is 165 m above sea level and the average rainfall 600 mm/year, most of it falling in the winter. The soil is a deep black vertisol with 42-49% clay, a high level of organic matter (C/N=11) and a pH of 8.0.

In 1979-80, wilt symptoms appeared in a corner of a 5 ha block sown with chickpea. The symptoms developed in patches in the local landrace, Amdoun, planted in March 1979. A 1 ha plot, which showed high wilt incidence, was delimited and the diseased chickpea plants incorporated into the soil by ploughing. During the following three years, the same variety was planted in the plot. Plants were protected against *Ascochyta* blight with chlorothalonil (Bravo 50 WP) applied as foliar sprays at the rate of 3 l/ha and at 7 to 10 day intervals. At the end of June of each year all plants were incorporated into the soil by ploughing.

In 1989 and 1990, the plot was divided into 25 squares of 10 m side and samples (500 g; 3 to 4 per square) were mixed. From this a composite sample of 100 g for each square was selected for determination of the number of colony forming units (cfus) by means of the dilution plate method and using Papavizas's selective medium (Papavizas,

1967; Appendix 2).

3.2.2. Plant material

In total, 1,915 chickpea varieties were kindly provided by Dr M.C. Saxena, Legume Programme, International Center for Agricultural Research in the Dry Areas, Aleppo, Syria (ICARDA). They were part of the world germplasm collection of "kabuli" chickpea which is maintained at this centre. About 100 seeds per variety were treated with benomyl (Benlate 50 WP) at the rate of 3 g/kg and thiobendazole (Tecto 60 WP) at the rate of 2.5 g/kg. All seed was kept at 4°C and 45-50% relative humidity.

3.2.3. Field screening

Owing to limitations of space in the WSP, the ILC varieties were screened in two batches. The first batch composed of 900 varieties was tested during the spring of 1989 and the second batch of 1,015 varieties during the spring of 1990. One week before sowing, the plot was disc-ploughed to remove weeds. At the same time, 100 kg of diammonium phosphate (DAP) and 20 kg of nematicide (Furadan) were incorporated into the soil, followed by a shallow harrowing. For planting, furrows were opened by a shallow furrow opener (10 to 14 cm depth) and seeds were dropped by hand into the bottoms of the furrows and covered with about 7 cm of soil.

For each variety, 40 seeds were sown in a single row, 4 m long, in two replicates, using a randomized block design. The inter-row spacing was 50 cm and the distance

between seeds was 10 cm. Two rows of test varieties were alternated with a single row of the wilt susceptible variety ILC 482 to serve as a reference and to maintain fungal inoculum in the plot. Planting was performed in the first week of March in both years (1989 and 1990) in strips separated by 2 m alleys.

Plants were protected against *Ascochyta* blight (see section 3.2.1) and leaf miner and pod borer were controlled by spraying the plants with an insecticide (Decis E45) at appropriate times. A manually operated sprayer was used to apply the fungicide and a tractor-mounted sprayer was used for insecticide application. The alleys were used as tracks for the tractor and all sprayers were calibrated to apply 350-400 liters of water per hectare. Weeds were removed by hand.

3.2.4. Wilt incidence

Base stand count was performed 5 to 7 days after emergence and data on wilt incidence was recorded 14 days after emergence and at four successive intervals of two weeks (D1, D2, D3, D4 and D5). It usually took 2 to 3 days to complete one rating and precautions were taken to make sure that every variety was scored at 14 days intervals. The dates of the first scorings were March 23 in 1989 and March 28 in 1990. Wilt incidence (WI) was calculated for each rating date as the total number of severely wilted or dead plants/total number of plants at base stand count x 100. Disease incidence was averaged for each variety over

the two replicates.

Seed of varieties which showed less than 10% mortality was harvested and stored at 4°C for future use.

3.3. RESULTS

3.3.1. Uniformity of the disease in WSP

The WSP had an acceptable degree of uniformity as shown by the numbers of cfus/g of soil of FOC from the 25 sampled squares (1795±253) and the reaction of the repeated check ILC 482 (Fig. 6). Since analysis of variance of wilt incidence data recorded for ILC 482 during 1989 and 1990 showed a non-significant level for the interaction cultivar x years, the data of all test varieties obtained during both years was treated as a single experiment.

3.3.2. Disease incidence and classification of varieties

Chickpea varieties differed significantly at all rating times. The analysis of variance performed on the WI data collected in both years showed highly significant effects ($P = 0.01$) owing to varieties. The block treatment did not show any significant effect indicating that varieties reacted similarly in both replicates.

In total, 110 lines had a resistant reaction (WI ≤10%). This represents 5.7% of the total number of tested varieties and these are characterised thoroughly in Chapter 6.

Fig. 6:

Upper: View of the WSP of Béja experimental station. The green strips in the mid-ground are plants of a wilt resistant variety released for cultivation in Tunisia and called Amdoun1.

Lower: Screening chickpea varieties for *Fusarium* wilt resistance in the WSP. Cholorotic rows are the susceptible check ILC 482.

The most dramatic variation in variety reaction was in the time of onset of symptoms (Fig. 7). At D1 (14 days after emergence) no variety, including the susceptible check ILC 482 showed symptoms. At D2 (28 days after emergence) 66 lines (3.5% of the total) showed 100% mortality with symptoms consisting of stunting, epinasty of leaves and stems and occasionally chlorosis. Such plants frequently collapsed and were termed "highly susceptible".

At later scoring dates a wide range of reactions was observed. The most striking ones were those varieties which were healthy at one scoring date but had 100% mortality by the next.

The different reactions which are presented in Table 7 were designated as follows:

1. Highly susceptible varieties = those which had 100% WI at D2 (28 days after emergence).

2. Very early wilters (VEW) = susceptible varieties which started to wilt at D2 and were completely wilted by D3 (42 days after emergence).

3. Early wilters (EW) = susceptible varieties which started to wilt at D3 and were completely wilted at D4 (56 days after emergence).

4. Late wilters (LW) = susceptible varieties which started to wilt at D4 and were completely wilted at D5 (70 days after emergence).

5. Very late wilters (VLW) = susceptible varieties which started to wilt or had 100% WI at D5.

Fig. 7:

Upper: Early wilting symptoms observed on chickpea varieties (**middle row**).

Lower: Symptoms of early wilting (**middle row foreground**),late wilting (**right row foreground**) and intermediate wilting (**left row foreground**). Very late wilting varieties (**medium green color**) are shown in the background of the photo.

Table 7

Fusarium wilt incidence observed at different times after emergence for different classes of chickpea varieties tested in a wilt sick plot at Beja. Tunisia

Classes of chickpea varieties	Wilt incidence (%)[1] at				Number of varieties	% of total number of screened varieties
	28 days D2	42 days D3	56 days D4	70 days D5		
Highly susceptible varieties	100	100	100	100	66	3.5
Very early wilters	11-99	100	100	100	89	4.6
Early wilters	0	11-100	100	100	179	9.3
Late wilters	0	0	11-100	100	248	12.9
Very late wilters	0	0	0	11-100	100	5.2
Resistant varieties	0	0	0	0	110	5.7
Slow wilting varieties	-	-	-	-	1123	56.5
Total number of varieties	-	-	-	-	1915	-

$$(1) = \frac{\text{Cumulative number of wilted or dead plants}}{\text{Total number of plants at stand count}} \times 100$$

- Wilt incidence figures are different from those in the other classes

6. Resistant varieties = those which showed less than 10% mortality at harvest time.

A residual of 1123 varieties were slow wilters which did not fit comfortably into the above classes (Fig. 8).

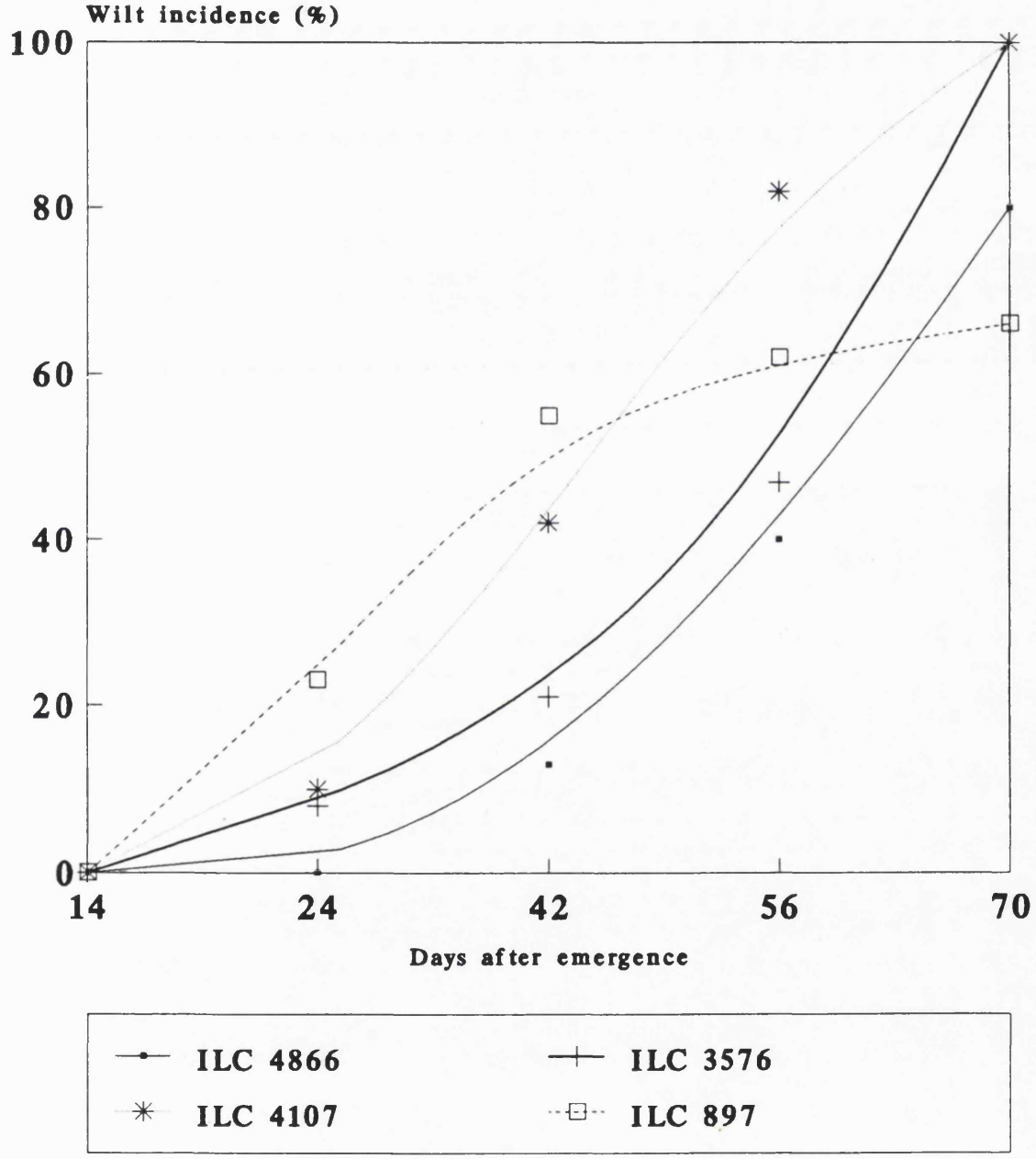
3.4. DISCUSSION

The wilt sick plot developed at Béja station was sufficiently uniform and well infested with FOC to provide appropriate disease pressure for screening chickpea lines (Fig. 6). Similar WSPs have been developed in other parts of the world and used successfully such as those at Santaella, Cordoba, Spain (Jiménez-Diaz *et al.*, 1991) and ICRISAT Center, Hyderabad, India (Nene *et al.*, 1981).

In the present study there were clear differences among varieties in the timing of the onset of symptoms and in disease progression. Seven classes were recognised and in six of these, once wilt symptoms were initiated, all plants succumbed within a short time. However, they were differentiated on the basis of timing of the onset of symptoms (Table 7). The seventh class contained varieties that were slow wilters (Fig. 8).

Although there is a genetic basis for this behaviour (section 1.7) the mechanism by which it is effected is not known. Various possibilities may be entertained. For example, the differences may be related to the ability of the pathogen to grow in the rhizosphere of the plant, the resistance of the plant root to penetration and the

Fig. 8: Wilt incidence (%) versus time for four chickpea varieties showing slow wilting



resistance of the plant conducting tissue to colonization. Alternatively, the differences may be related to the ability of the pathogen to elaborate toxic compounds and the relative sensitivity of the cultivars to these substances. This hypothesis is particularly attractive for cultivars that wilt early and suddenly. A further possibility is related to the ability of the plant to compensate for roots that are already diseased by the production of new roots (Huisman, 1982). Genotypic differences in rooting have been observed between varieties (Singh *et al.*, 1980; Gregory, 1988).

CHAPTER 4

DEVELOPMENT OF *FUSARIUM* WILT OF CHICKPEA IN CONTROLLED ENVIRONMENT EXPERIMENTS

4.1. INTRODUCTION

Air temperature and inoculum density are likely to be important factors in the expression and development of *Fusarium* wilt of chickpea but at the start of this work there were no published reports on their roles. Recently, however, data from experiments conducted under controlled conditions were published by Bhatti and Kraft (1992a). They observed severe wilt symptoms at 25°C and 30°C whereas no disease developed at 10°C even at high inoculum densities.

Van Rheenen *et al.* (1989) pointed out the importance of inoculum density in the development of *Fusarium* wilt. They reported that an inoculum density (ID) of 67 to 483 propagules/g of soil caused 100% mortality in susceptible cultivars at ICRISAT Centre, Hyderabad, India. Bhatti and Kraft (1992a) found that an ID of 500 colony-forming units per gram of soil (cfu/g) is enough for FOC to be highly pathogenic to two susceptible varieties, Burpee 5043 and J6-62, providing the temperature was between 25 and 30°C.

In this chapter, experiments with a range of temperatures and inoculum densities with plants varying in susceptibility are reported.

4.2. MATERIALS AND METHODS

4.2.1. Fungal isolates and preparation of inoculum

Isolate FG_3 of FOC (section 2.3.1) was used. Inoculum production and preparation of inoculated and non-inoculated control soil were as described previously (section 2.2.5). Four levels of inoculum, 500±73, 1000±189, 2000±275 and 4000±463 colony-forming units per gram of air-dried soil (cfu/g), were prepared and adjusted by supplementing autoclaved soil with specific amounts of inoculated soil. The number of cfus was determined as described previously (section 3.2.1).

4.2.2. Cultivars, planting and air temperature régimes

Seed of the varieties ILC 223, ILC 482 and ILC 3279, respectively a very early wilter, an early wilter and a late wilter were planted in 15 cm plastic pots containing 800g of soil (section 2.2.5). The pots were placed in reach-in controlled environment chambers (Rumed, Rubarth Apparate GmbH, Hannover, West Germany) set at constant air temperatures: 10, 15, 20, 25 and 30±1°C. The relative humidity was maintained at 50% or less and a photoperiod of 14 h fluorescent light at approximately 250 $\mu E\ m^{-2}\ s^{-1}$ at plant height was imposed. The pots were watered as necessary with distilled water, and fertilised weekly with 100 ml of nutrient solution (Hoagland, 1950). They were observed daily for wilt symptoms and numbers of wilted plants were recorded. Wilt incidence, as a percentage, was computed. Plants were considered wilted when drooping of

leaves (epinasty) either associated or not with yellowing was noticed. For each variety the experiment was a split plot design with temperature as the main treatment and inoculum density as the second level treatment. There were three replicates per treatment and 5 to 6 plants per pot. The pots were rearranged randomly in each growth chamber every 3-4 days.

4.2.3. Progress of wilt and changes in inoculum levels during wilt development

Plants of the varieties ILC 223, ILC 482 and ILC 3279 were grown (five per pot) in soils containing 1000 and 4000 cfu/g at 25°C. After 3 weeks, they were harvested at the soil line and the leaves removed. An outline of the plants was made on white paper and the stems were cut into 1 cm sections. The sections were surface sterilized in 2.5% sodium hypochlorite solution for 3 min, dried on filter paper and plated on PDA. Plates were incubated for 7 days at room temperature and observed for fungal growth. Sections of the stem in which pathogen developed were noted on the outline, thus giving a diagram of the progression of the fungus in the plant.

At the end of the experiment, pots from the 25°C regime were left at room temperature for 7 days before removing samples from a depth of 5-7 cm. The number of cfu/g of soil was determined as described earlier (section 3.2.1).

4.2.4. Statistical analysis

Analysis of variance and F-tests were performed to determine differences among cultivars, temperature régimes and inoculum levels. Disease progress curves as a function of temperature and inoculum density (ID) were fitted for each variety by linear regression and the t_{50} (time required to reach 50% wilt incidence) was calculated.

4.3. RESULTS

4.3.1. Effect of temperature and inoculum density on symptom initiation and development

4.3.1.1. Symptom initiation

The time taken to the first appearance of symptoms was influenced by inoculum density and temperature (Fig. 9). At 10°C no disease development occurred in any variety at any inoculum level but at 15°C and the highest inoculum level (4000 cfu/g), wilt symptoms appeared rapidly (at 11 days) in ILC 223 but more slowly in the other two varieties. At this inoculum level, the timing of the appearance of wilt symptoms in ILC 223 was similar at the other three temperatures (20, 25 and 30°C) but in the two varieties, ILC 482 and ILC 3279, increasing temperature decreased the time required for the onset of symptoms.

At lower inoculum levels, differences in the timing of the onset of symptoms in the three varieties at 15°C were less pronounced or non-existent (Fig. 9). The greatest differences among all three cultivars were at 20°C and inoculum levels of 500 or 1000 cfu/g. Plants in control

Fig. 9:

Effect of constant air temperature and inoculum level on number of days to the onset of wilt symptoms induced by FOC isolate FG_3 on chickpea varieties, ILC 223, ILC 482 and ILC 3279.

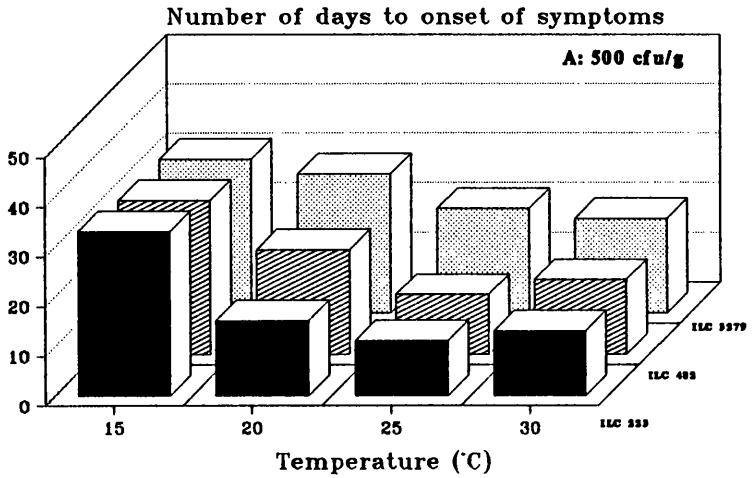
A: 500 cfu/g of soil.

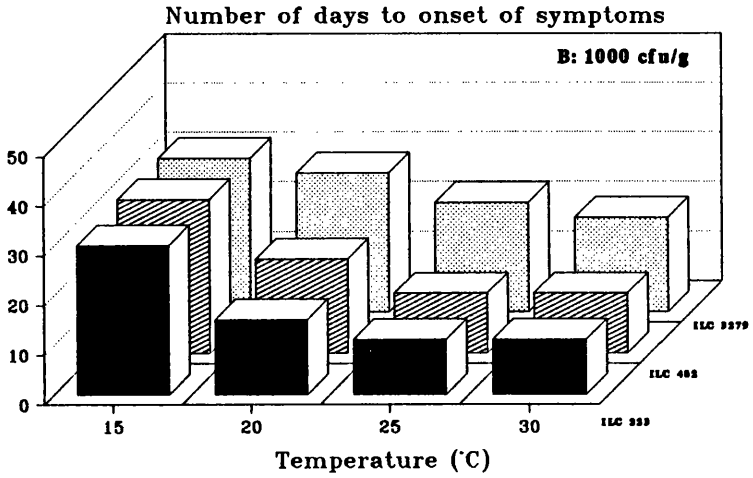
B: 1000 cfu/g of soil.

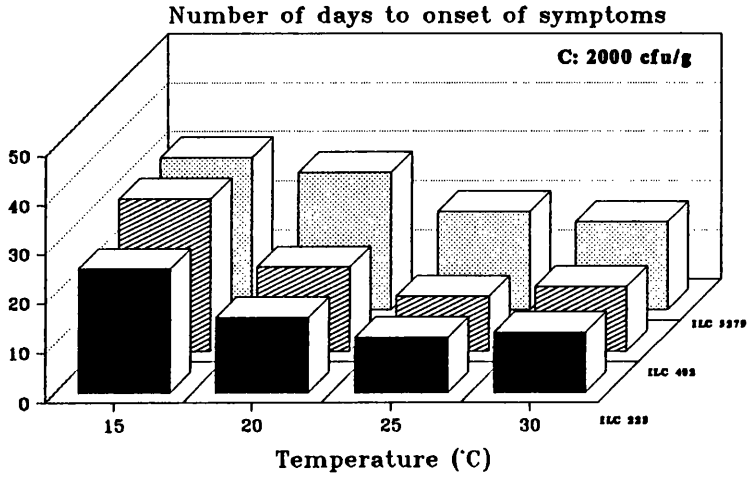
C: 2000 cfu/g of soil.

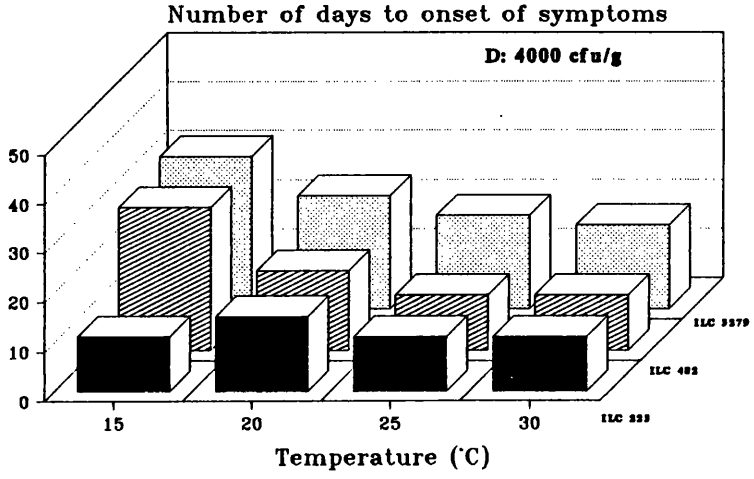
D: 4000 cfu/g of soil.

(Data tabulated in appendix 3)


Number of days to onset of symptoms
A: 500 cfu/g
50
40
30
20
10
0
15
20
25
30
Temperature (°C)


Number of days to onset of symptoms
B: 1000 cfu/g
50
40
30
20
10
0
15
20
25
30
Temperature (°C)


Number of days to onset of symptoms
C: 2000 cfu/g
50
40
30
20
10
0
15
20
25
30
Temperature (°C)


Number of days to onset of symptoms
D: 4000 cfu/g
50
40
30
20
10
0
15
20
25
30
Temperature (°C)

pots showed no symptoms.

4.3.1.2. Symptom development

The percentage wilt incidence was essentially linearly related to time at all inoculum levels and temperatures (except 10°C at which temperature no disease developed; Fig. 10). This allowed the time taken for 50% of the plants to wilt (t_{50}) to be derived (Fig. 11). These figures match the results obtained for the onset of symptoms (Fig. 9) except that the timings are, of course, greater.

In order to present the interaction of variety, inoculum level and temperature more clearly, the rate of disease progress for the three varieties was tabulated (Tables 8, 9 and 10) and plotted against temperature for the four inoculum levels (Fig. 12). Disease development was maximal at 25°C and at the highest inoculum levels for all three varieties. The distinction between the highest and lowest inoculum density was most pronounced in ILC 482 at 25°C, although there was no difference in the rate of disease progress between the two intermediate inoculum levels. Results for ILC 3279 were similar, although for the three highest inoculum levels the rate of disease progress was less than that for ILC 482.

4.3.2. Fungal colonization of the plant

Inoculum density had little effect on the extent of colonization of the plant. Surprisingly, the more

Fig. 10:

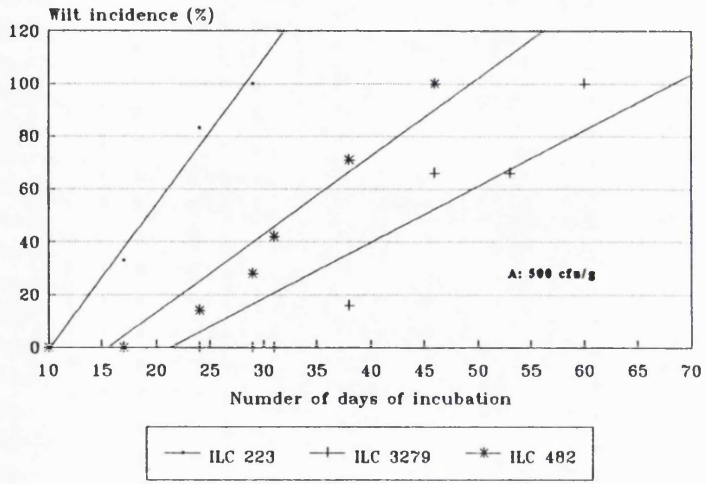
Idealized linear disease progress curves (Harvard Graphics package) for three chickpea varieties grown at 20°C and three inoculum levels.

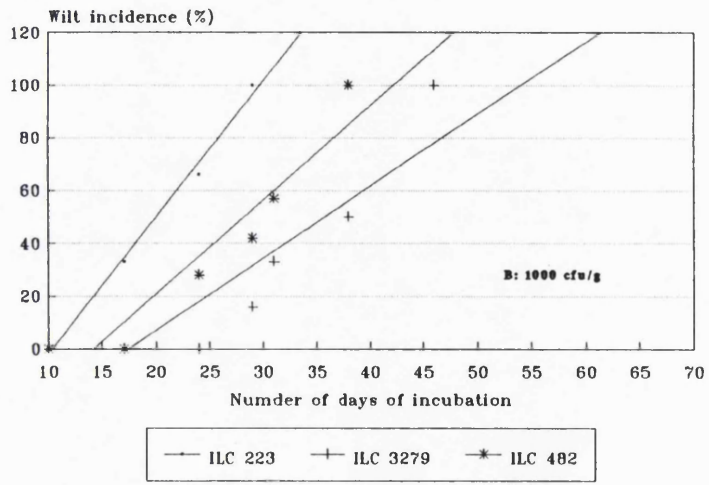
A: 500 cfu/g of soil

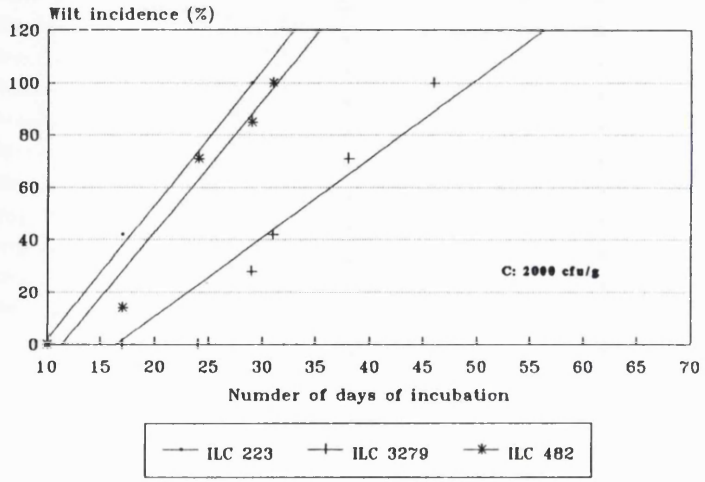
B: 1000 cfu/g of soil

C: 2000 cfu/g of soil

D: 4000 cfu/g of soil


Wilt incidence (%)
120
100
80
60
40
20
0
10 15 20 25 30 35 40 45 50 55 60 65 70
Numder of days of incubation
A: 500 cfu/g
ILC 223
ILC 3279
ILC 482


Wilt incidence (%)
120
100
80
60
40
20
0
10 15 20 25 30 35 40 45 50 55 60 65 70
Numder of days of incubation
B: 1000 cfu/g
ILC 223
ILC 3279
ILC 482


Wilt incidence (%)
120
100
80
60
40
20
0
10 15 20 25 30 35 40 45 50 55 60 65 70
Numder of days of incubation
C: 2000 cfu/g
ILC 223
ILC 3279
ILC 482

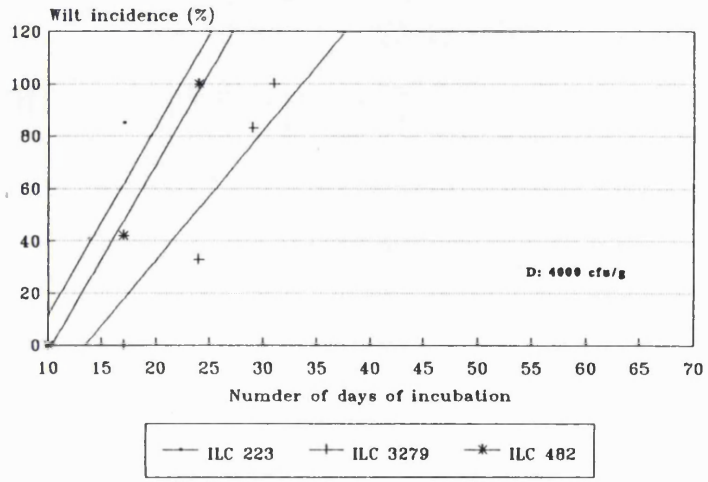

Wilt incidence (%)
120
100
80
60
40
20
0
10 15 20 25 30 35 40 45 50 55 60 65 70
Numder of days of incubation
D: 4000 cfu/g
ILC 223
ILC 3279
ILC 482

Fig. 11:

Effect of constant air temperature and inoculum level on number of days to 50% wilt incidence induced by FOC isolate FG_3 on chickpea varieties, ILC 223, ILC 482 and ILC 3279.

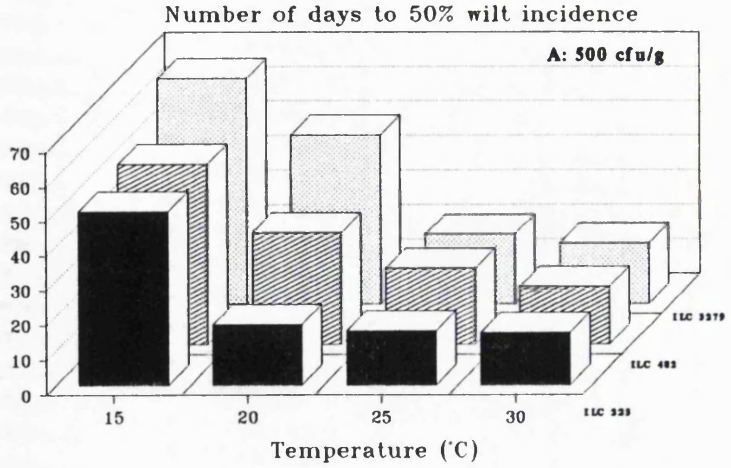
A: 500 cfu/g of soil.

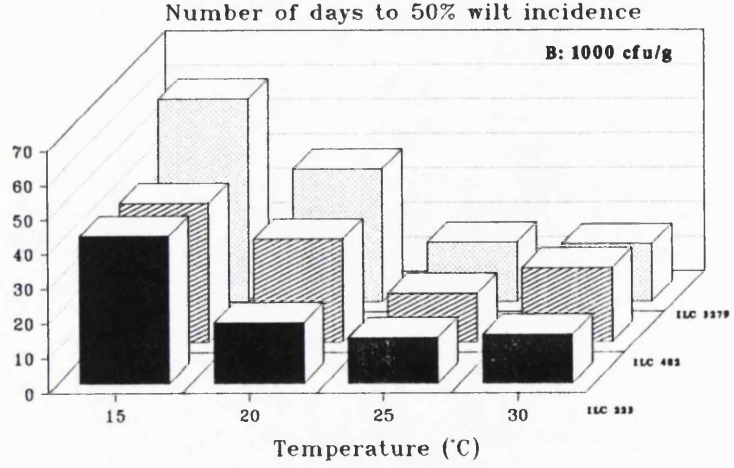
B: 1000 cfu/g of soil.

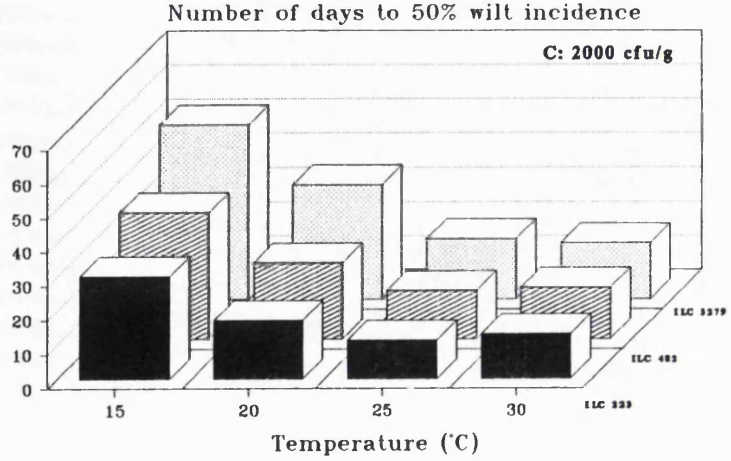
C: 2000 cfu/g of soil.

D: 4000 cfu/g of soil.

(Data tabulated in appendix 4)


Number of days to 50% wilt incidence
A: 500 cfu/g
70
60
50
40
30
20
10
0
15
20
25
30
ILC 3279
ILC 482
ILC 333
Temperature (°C)


Number of days to 50% wilt incidence
B: 1000 cfu/g
70
60
50
40
30
20
10
0
15
20
25
30
ILC 3279
ILC 482
ILC 333
Temperature (°C)


Number of days to 50% wilt incidence
C: 2000 cfu/g
70
60
50
40
30
20
10
0
15
20
25
30
ILC 3279
ILC 482
ILC 333
Temperature (°C)

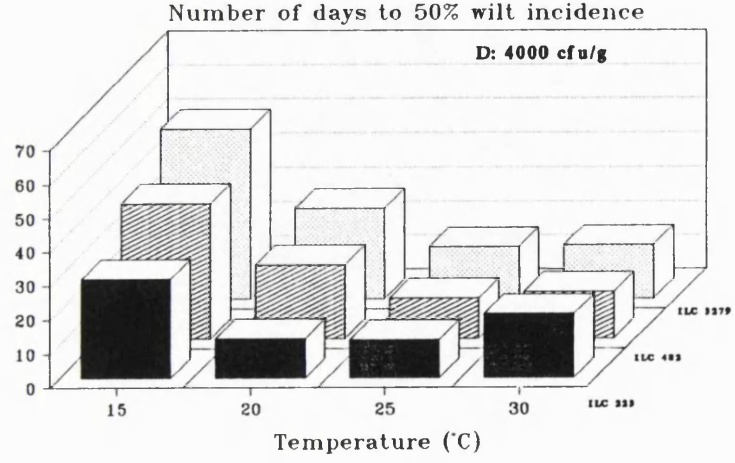

Number of days to 50% wilt incidence
D: 4000 cfu/g
70
60
50
40
30
20
10
0
15
20
25
30
ILC 3279
ILC 482
ILC 333
Temperature (°C)

Table 8

Comparison of statistics obtained from regression of wilt incidence on time for variety ILC 223 and for different combinations of air temperatures and ID levels.

Temperature and ID (cfu/g)	Intercept [1]	Slope[1]	R^2[2]	F test[3]
15°C				
4000	-23.83	2.53 a	53.8	**
2000	-26.75	2.56 a	71.2	**
1000	-18.44	1.61 ab	47.1	**
500	-17.61	1.35 b	49.2	**
20°C				
4000	-10.05	5.15 a	87.2	**
2000	-13.60	3.74 a	93.1	**
1000	-14.98	3.76 b	84.5	**
500	-20.91	4.10 b	80.1	**
25°C				
4000	-32.00	7.32 a	65.3	**
2000	-32.27	7.30 a	71.4	**
1000	-29.09	6.09 a	68.8	**
500	-25.60	4.92 b	74.5	**
30°C				
4000	-32.22	6.92 a	69.7	**
2000	-28.06	5.94 b	61.7	**
1000	-25.37	5.48 b	56.9	**
500	-20.63	4.71 c	49.5	**

(1) Intercept and slope are parameters of linear regression equations computed on non-transformed data.
(2) R^2: Coefficient of determination.
(3) **: Significant at P = 0.01

Table 9

Comparison of statistics obtained from regression of wilt incidence on time for variety ILC 482 and for different combinations of air temperatures and ID levels.

Temperature and ID (cfu/g)	Intercept [1]	Slope[1]	R^2[2]	F test[3]
15°C				
4000	-20.31	1.76 ab	47.2	**
2000	-25.36	2.01 a	81.2	**
1000	-24.47	1.85 ab	81.4	**
500	-17.81	1.30 ab	83.5	**
20°C				
4000	-41.83	4.25 a	59.6	**
2000	-41.82	4.11 a	70.8	**
1000	-35.00	2.85 b	76.9	**
500	-39.81	2.78 b	89.1	**
25°C				
4000	-28.80	6.67 a	53.3	**
2000	-28.18	5.51 b	68.6	**
1000	-27.53	5.57 b	69.6	**
500	-21.96	3.30 c	81.2	**
30°C				
4000	-29.66	5.79 a	67.6	**
2000	-27.48	5.10 a	65.7	**
1000	-18.25	3.92 b	45.6	**
500	-24.31	4.50 a	58.1	**

(1) Intercept and slope are parameters of linear regression equations computed on non-transformed data.
(2) R^2: Coefficient of determination.
(3) **: Significant at P = 0.01

Table 10

Comparison of statistics obtained from regression of wilt incidence on time for variety ILC 3279 and for different combinations of air temperatures and ID levels.

Temperature and ID (cfu/g)	Intercept (1)	Slope(1)	$R^{2(2)}$	F test(3)
15°C				
4000	-16.49	1.33 a	88.7	**
2000	-17.15	1.31 a	86.5	**
1000	-15.47	1.11 a	82.4	**
500	-14.69	0.99 a	78.3	**
20°C				
4000	-45.05	3.54 a	57.6	**
2000	-42.88	2.76 a	81.2	**
1000	-37.65	2.28 a	77.4	**
500	-34.79	1.73 b	75.7	**
25°C				
4000	-30.71	5.30 a	72.2	**
2000	-25.80	4.24 a	71.6	**
1000	-26.62	4.48 a	75.1	**
500	-21.94	3.57 b	69.0	**
30°C				
4000	-38.16	4.64 a	61.8	**
2000	-36.00	4.30 a	61.2	**
1000	-45.16	4.51 a	67.1	**
500	-36.34	4.14 a	72.7	**

(1) Intercept and slope are parameters of linear regression equations computed on non-transformed data.
(2) R^2: Coefficient of determination.
(3) **: Significant at P = 0.01

Fig. 12:

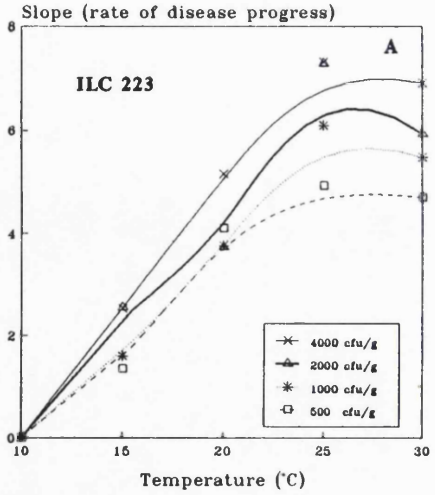
Disease progress curves as described by the slope [1] of the regression of wilt incidence on time for three varieties grown at five temperatures and four inoculum levels of FOC.

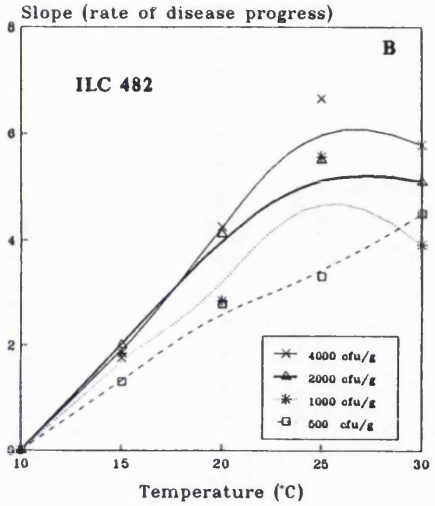
A: ILC 223

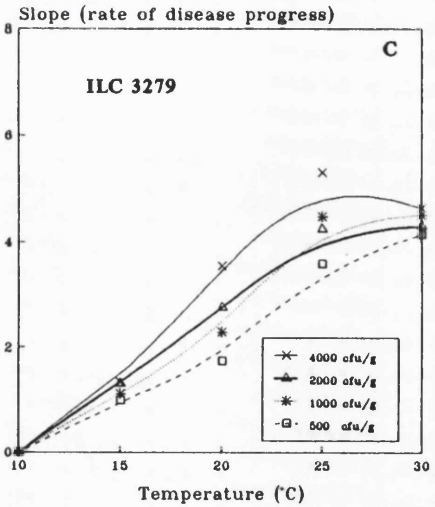
B: ILC 482

C: ILC 3279

(1) The slope is the rate of disease progress expressed as the % wilted plants/day.


Slope (rate of disease progress)
A
ILC 223
4000 cfu/g
2000 cfu/g
1000 cfu/g
500 cfu/g
Temperature (°C)


Slope (rate of disease progress)
B
ILC 482
4000 cfu/g
2000 cfu/g
1000 cfu/g
500 cfu/g
Temperature (°C)


Slope (rate of disease progress)
C
ILC 3279
4000 cfu/g
2000 cfu/g
1000 cfu/g
500 cfu/g
Temperature (°C)

resistant varieties ILC 482 and ILC 3279 were colonized to a greater extent than the more susceptible variety ILC 223 (Fig. 13).

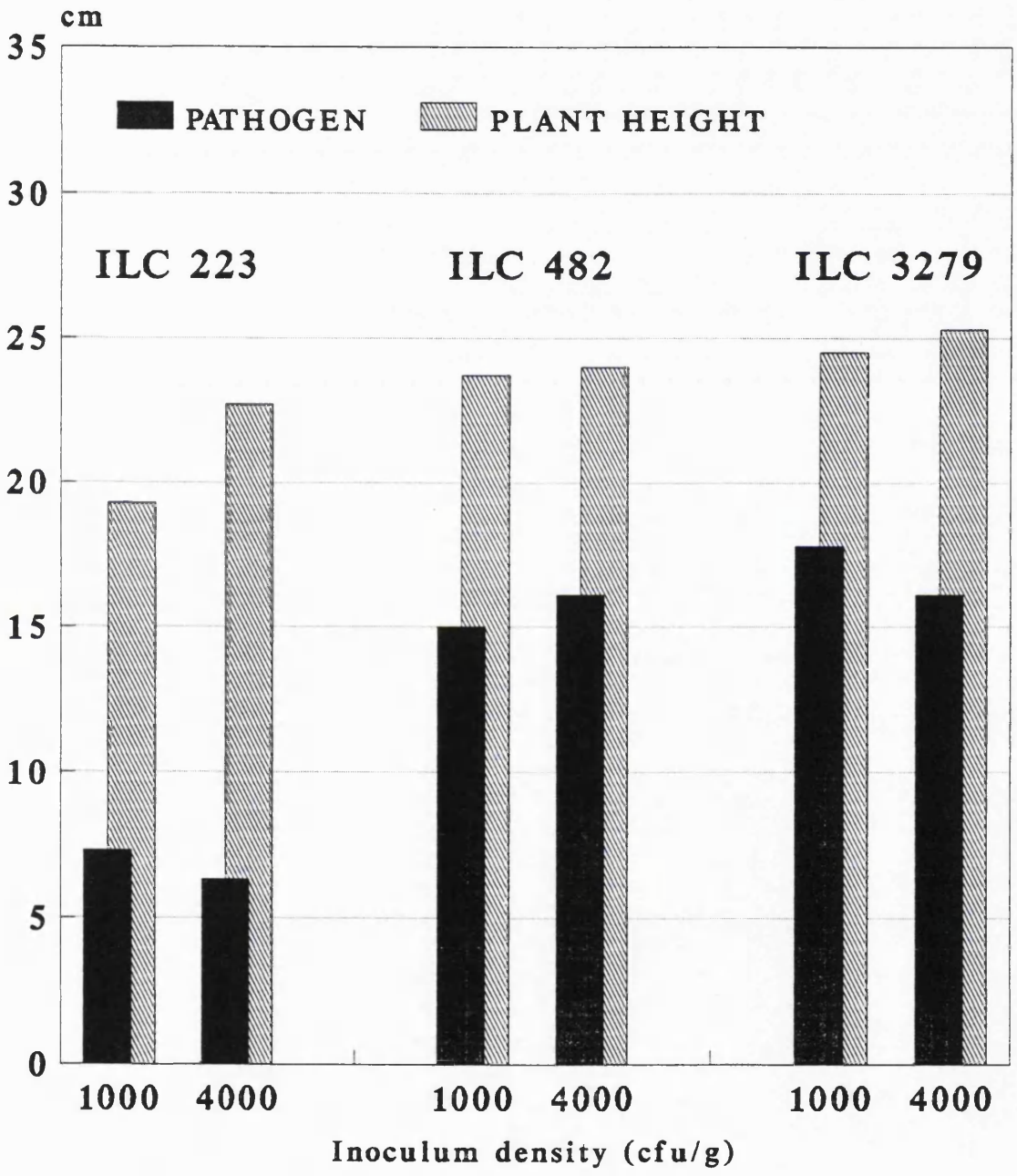
4.3.3. Multiplication of the fungus during growth of plants

Little variation in the number of cfus occurred during the course of the experiment when the varieties ILC 482 and ILC 3279 were grown in soil initially containing 4000 cfu/g. In contrast, there was a reduction in cfus in soil planted with the more susceptible cultivar ILC 223. At an initial level of 2000 cfu/g there was no change in soil planted with variety ILC 223 but there was an increase in soil planted with the other two varieties. In soil planted with all three varieties, cfus increased when the original levels were initially 1000 or 500 cfu/g (Table 11).

4.4. DISCUSSION

In this study, in agreement with the results of Bhatti and Kraft (1992a), no disease occurred at 10°C. At the four higher temperatures wilt developed in the three varieties. These varieties, ILC 233, ILC 482 and ILC 3279 were classified as very early, early and late wilters, respectively (section 3.3.2 and 5.3.1). This differentiation was most evident in the initiation of symptoms at a temperature of 20°C and inoculum levels of 500 and 1000 cfus/g (Fig. 9) and these results were also

Fig. 13: Extent of colonization of three chickpea varieties inoculated with FOC and grown for three weeks at 25 C.



(Data tabulated in appendix 5)

Table 11

Initial and final numbers of cfus of FOC when plants were grown in infested soil for three weeks at 25°C.

Initial ID levels (cfu/g of soil)	Levels of cfu/g at the end of the experiment		
	ILC 223	ILC 482	ILC 3279
4000 ± 463	3260 ± 311	4120 ± 299	3980 ± 405
2000 ± 275	1808 ± 167	2981 ± 150	2670 ± 213
1000 ± 189	1717 ± 189	2070 ± 312	1856 ± 123
500 ± 73	1375 ± 219	1259 ± 224	989 ± 211
0	0	0	0

reflected in the t_{50} values (Fig. 11).

In agreement with the results of Cabrera de La Colina et al (1988), high inoculum densities promoted wilt symptom expression and progression. However, Van Rheenen *et al.* (1989) reported that a threshold of 67 to 483 propagules/gram of soil caused 100% mortality in susceptible varieties. Unfortunately, neither the time of the observations nor the temperature of the experiment were reported. At 20°C and all inoculum levels all plants eventually succumbed but they did so at different times according to their wilt classification (Fig. 10). These results are in agreement with those reported for other wilt diseases. For example, Welch (1981) observed an increase in infection incidence at higher inoculum densities of *Fusarium oxysporum* f. sp. *apii* and related this to greater excretion of exudates from diseased roots that stimulate additional germination of spores.

Other authors have stressed the importance of temperature in the development of chickpea wilt. For example, Bhatti and Kraft (1992b) reported that temperatures of 25°C and 30°C were optimal for symptom development but Gupta *et al.* (1987) found that at 30°C symptom development was significantly decreased. In the present experiments wilt development, in agreement with the work of Bhatti and Kraft (1992b) was similar at the two temperatures (Fig. 12). These temperatures are similar to those reported by Gardiner *et al.* (1987) for chrysanthemum wilt caused by *Fusarium oxysporum* f. sp.

chrysanthemi and tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Clayton, 1923).

Surprisingly, fungal colonization of the plant was less in the very early wilter ILC 233 than the other two cultivars (Fig. 13). In the field this variety suddenly collapses within 10 to 15 days of germination. One possible explanation for this violent syndrome could be sensitivity to toxins produced by the fungus and this is considered further in Chapter 7. Jiménez-Diaz *et al.* (1989a) showed that at 26°C race 5 colonized the plant faster than race 0 and colonization was more intense in the root and the lower part of the stem resulting in occlusion of xylem vessels.

Evaluation of the cfus of the fungus showed that the pathogen multiplied during the course of the experiment when the initial levels were low (Table 11). Van Rheenen *et al.* (1989) also reported an increase in FOC propagules during the development of wilt in the field at ICRISAT, India. These findings may be important in the epidemiology of the disease.

CHAPTER 5

DEVELOPMENT OF *FUSARIUM* WILT OF CHICKPEA IN FIELD EXPERIMENTS

5.1. INTRODUCTION

Disease development and expression of symptoms of *Fusarium* wilt under field conditions are influenced by isolate of the pathogen, variety of the host and the environment. The latter includes both physical factors such as temperature, soil type and soil moisture and biotic factors such as soil microflora, inoculum density and plant age (Jiménez-Diaz *et al.*, 1990c). Reports of field research work on the role of these factors are few and therefore they were studied under Tunisian conditions.

5.2. MATERIALS AND METHODS

5.2.1. Plant genotypes

The varieties selected for testing are popular or are in the process of becoming popular in the WANA region and have been adopted for cultivation by several National Programmes (ICARDA, 1991). They were all susceptible to *Fusarium* wilt but differed in their time to the onset of the disease (sections 3.3.2 and 4.3.1; Halila, unpublished data). The varieties and their various designations were as follows:

1. ILC 482: released in Morocco under the same name, in France as TS 1009, in Jordan as Jubeiha-2, in Lebanon as Janta 2, in Syria as Ghab 1 and in Turkey as Guney Sarisi.
2. ILC 3279: released in Algeria under the same name, in Cyprus as Yialousa, in Italy as Sultano, in Jordan as Jubeiha-3, in Syria as Ghab 2 and in Tunisia as Chitoui.
3. FLIP 83-46C: released in Tunisia as Kassab.
4. FLIP 84-79C: this variety is in the pre-release process in Algeria and Tunisia.

Seed was multiplied by the Tunisian National Programme and good quality samples were treated with Benlate (Benomyl 50 WP) and conserved at 4°C and 40-50% relative humidity.

5.2.2. Planting

All trials took place in the wilt sick plot (WSP) of Béja experimental station during the springs of 1988, 1989, 1990, 1991 and 1992. Seedbed preparation, planting procedure and phytosanitary protection were performed as previously described (sections 3.2.1 and 3.2.3). Planting dates varied from year to year but were always within the first two weeks of March.

Plants were sown in single rows 4 m long and 50 cm apart, using a ramdomized block design essentially as described in section 3.2.3 except that 50 seeds were planted in each row. For each variety, the number of

replicates varied from one season to an other, with a minimum of 10 in 1988 and a maximum of 16 in 1992.

5.2.3. Disease progress assessement

Plants were observed regularly from 1 week after emergence and the numbers that were dead were recorded as a percentage of the stand count (taken at 5-7 days after emergence). The number of observations during the growing season varied between five and nine depending on the year of the experiment. At each scoring date, 2-3 dead plants, chosen at random, were checked for vascular discoloration.

5.2.4. Monitoring of environmental variables

Environmental data were recorded daily during the growing seasons at the weather station located in the field plots of Béja experimental station as follows:-

X1 = cumulative number of days having an average air temperature ≥20°C.

X2 = cumulative number of days with average air temperature ≥25°C.

X3 = cumulative number of days with soil temperature at 10 cm depth ≥20°C.

X4 = cumulative number of days with soil temperature at 20 cm depth ≥20°C.

X5 = cumulative number of days having a maximum air temperature ≥25°C.

X6 = cumulative number of days having a maximum air

temperature <25°C.

X7 = cumulative number of growth degree days (GDD) (base = 0°C).

X8 = cumulative number of degree days (base = 0°C) calculated for days with an average air temperature ≥25°C (GDD ≥25°C).

X9 = cumulative number of degree days (base = 0°C) calculated for days with an average air temperature <25°C (GDD < 25°C).

X10 = soil degree days at 10 cm depth (base = 0°C).

X11 = soil degree days at 20 cm depth (base = 0°C).

X12 = soil degree days at 10 cm depth for days with soil temperature ≥25°C (base = 0°C).

X13 = Summation of maximum air temperatures which were ≥25°C.

The 20 and 25°C temperatures were selected on the basis of results recorded in chapter 4 and on previous personal observations on the progress of *Fusarium* wilt. Environmental parameters were recorded daily from the time of planting.

5.2.5. Data analysis

In order to analyze the relations between disease development and environmental variables, multiple regression equations were developed using the SAS statistical package (SAS, 1985) with the PROG REG option and a FORWARD selection set at P = 0.05. The FORWARD

selection technique begins with no variables in the model and the variables are added one by one and stay there if their F statistics have a significance level greater than the P value (SAS, 1985).

5.3. RESULTS

5.3.1. A comparison of disease progress in the four varieties over 5 years

The untransformed data of percent wilt plotted against time generally gave a good approximation to a straight line as shown by the coefficient of determination (Table 12). Considerable differences in the time of onset of symptoms and rate of disease increase occurred among the four varieties. In all five years this was fastest in ILC 482 and slowest in FLIP 84-79C (Fig. 14; Table 12). This is in accord with their designation as early and very late wilter, respectively (Chapter 3). The other two varieties were intermediate in years 1989 and 1990 but were closer to ILC 482 than FLIP 84-79C in 1988 and closer to FLIP 84-709C than ILC 482 in 1991 and 1992. These results, as well as inspection of the graphs (Fig. 14) showed the strong influence of year on disease progress. In 1989, ILC 482 was the only variety to wilt completely, whereas in 1990 and 1992 all varieties were 100% wilted by the end of the season except FLIP 84-79C where the figures were 78% in 1990 and 97% in 1992. All wilted plants that were sampled showed discoloration of the vascular system.

When the data for t_{10}, t_{50} and t_{90} for all 5 years were

Table 12

Statistics obtained from regression of wilt incidence on time for four chickpea varities tested in the Béja WSP during 1988-92.

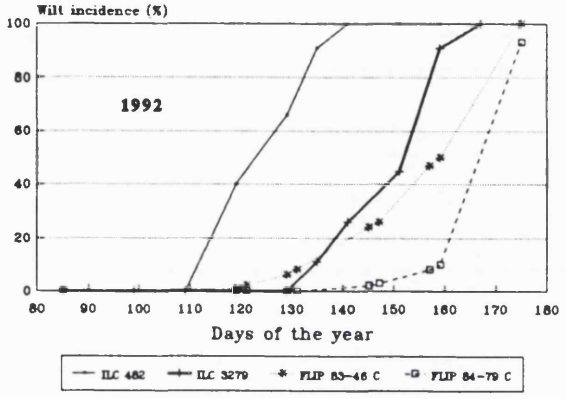
Variety and year	Disease progress rate (Slope)	Coefficient of determination (R^2)	t_{10} (2)	t_{50} (2)	t_{90} (2)
ILC 482					
1988	2.43 b	0.89*(1)	34	51	67
1989	2.40 b	0.96*	27	44	61
1990	2.73 b	0.63	16	31	45
1991	4.46 a	0.68	27	47	45
1992	2.80 b	0.94*	33	47	62
Five years combined	2.96		27.0	41.8	55.9
ILC 3279					
1988	2.21 a	0.96*	37	55	73
1989	0.99 c	0.67	43	84	124
1990	3.49 b	0.92*	25	36	48
1991	1.40 a	0.77	43	72	100
1992	1.59 a	0.80**	47	72	97
Five years combined	1.93		39.0	63.8	88.5
FLIP 83-46 C					
1988	2.19 b	0.98**	38	57	75
1989	1.00 c	0.70	43	83	123
1990	3.73 a	0.96*	29	40	50
1991	1.68 b	0.77**	39	63	87
1992	1.67 b	0.92**	45	68	91
Five years combined	1.99		38.8	62.2	85.2
FLIP 84-79 C					
1988	0.69 b	0.96**	54	112	169
1989	0.33 c	0.74*	64	185	306
1990	2.86 a	0.80	34	48	62
1991	0.82 b	0.65**	49	98	147
1992	1.46 b	0.63	53	80	107
Five years combined	1.19		50.8	104	158

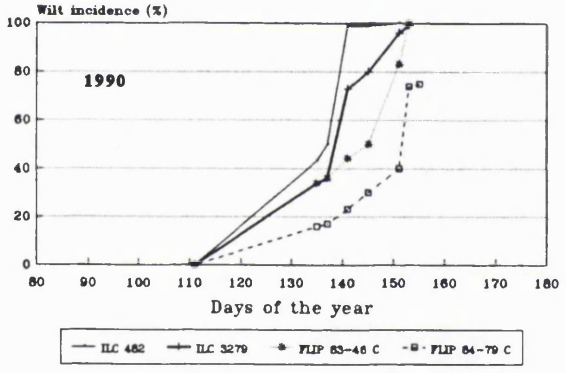
(1) * Significant at P = 0.05
** Significant at P = 0.01

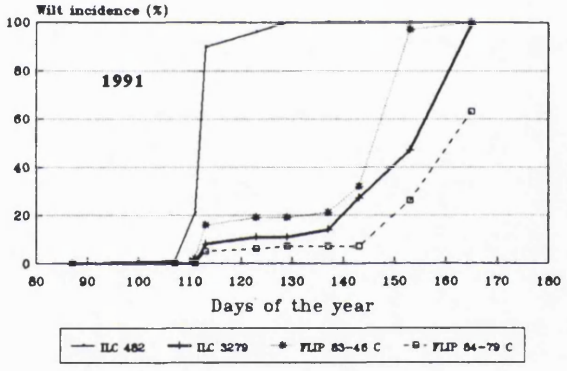
(2) computed by extrapolation using the developed regression equations, t_{10}, t_{50} and t_{90} are number of days for wilt incidence to reach 10%, 50% and 90% respectively

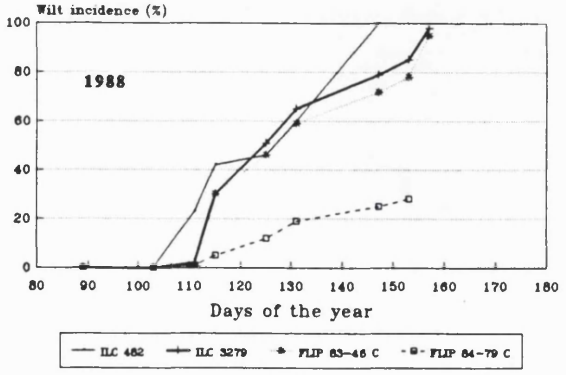
Fig. 14:

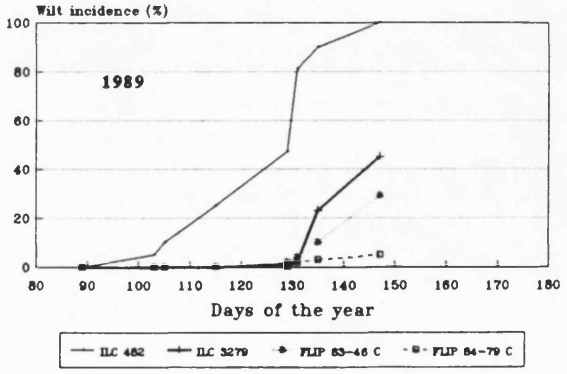
Wilt disease progress curves for four chickpea varieties, ILC 482, ILC 3279, FLIP 83-46C and FLIP 84-79C planted during five growing seasons (1988-1992) in the wilt sick plot of Béja experimental station.


Wilt incidence (%)
1992
Days of the year
ILC 482
ILC 3279
FLIP 83-46 C
FLIP 84-79 C


Wilt incidence (%)
1990
Days of the year
ILC 482
ILC 3279
FLIP 83-46 C
FLIP 84-79 C


Wilt incidence (%)
1991
Days of the year
ILC 482
ILC 3279
FLIP 83-46 C
FLIP 84-79 C


Wilt incidence (%)
1988
Days of the year
ILC 482
ILC 3279
FLIP 83-46 C
FLIP 84-79 C


Wilt incidence (%)
1989
Days of the year
ILC 482
ILC 3279
FLIP 83-46 C
FLIP 84-79 C

averaged the designations of ILC 482 as an early wilter and ILC 3279 as a late wilter were confirmed. FLIP 83-46C was similar to ILC 3279 and FLIP 84-79C appeared to be a very late wilter (Fig. 15).

5.3.2. Relationships between disease progress and environmental variables

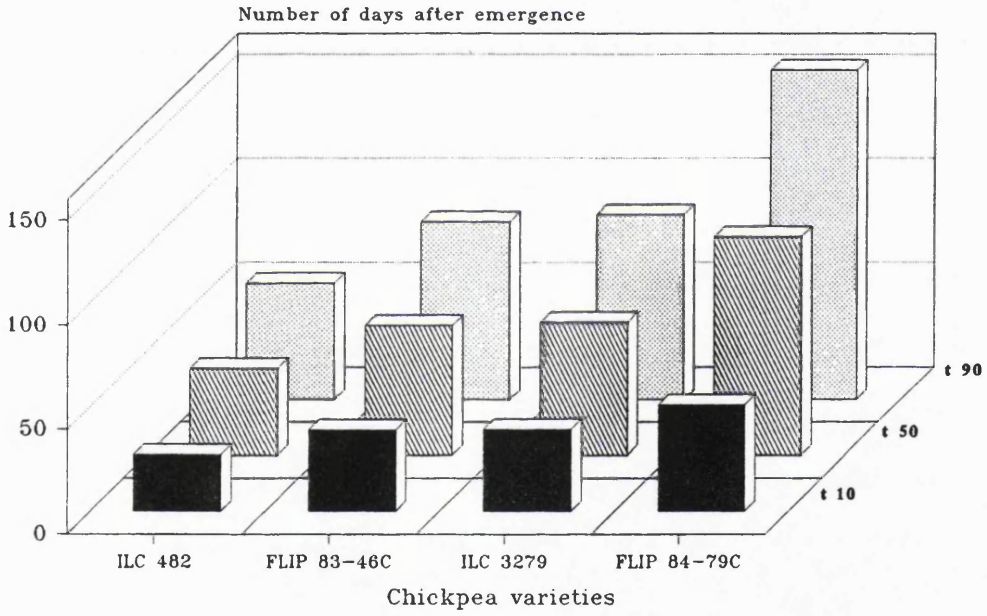
Multiple regression models associating disease progress and 13 different environmental variables were developed. The 13 variables could not be analysed together with the multiple regression models owing to lack of the required degrees of freedom. For this reason different computer programs were written for small homogeneous groups (G) of variables. These groups were:

1. G1, composed of variables X7, X8, X9 and X13 which affect the growth of chickpea plant.
2. G2, composed of variables X10, X11 and X12. These are related to the effect of soil environment on chickpea growth.
3. G3, composed of variables X1, X2, X5 and X6 which are related to air temperature.
4 G4, composed of soil temperature variables X3 and X4.

5.3.2.1. Air temperature variables (G1 and G3)

Tables 13 and 14 show that high air temperature is the variable most often associated with disease progression. Variables differed from year to year and from

Fig. 15: Number of days to reach 10% (t10), 50% (t50) and 90%(t90) wilt incidence for four chickpea varieties (1).



(1) average of five years

Table 13

Results of forward multiple regression between *Fusarium* wilt incidence of 4 chickpea varieties and (G1) environmental variables [2] X7, X8, X9 and X13 collected during the experimental period 1988-1992

Variety/ Year	ILC 482		FLIP 83-46 C		ILC 3279		FLIP 84-79 C	
	Variable	$R^{2(1)}$	Variable	R^2	Variable	R^2	Variable	R^2
1988	X_9	0.90	X_{13}	0.79	X_{13}	0.93	X_7	0.94
1989	X_{13}	0.97	X_{13}	0.90	X_{13}	0.84	X_{13}	0.80
1990	X_8	0.91	X_9	0.93	X_{13}	0.72	X_9	0.92
1991	X_9-X_8	0.95	X_7	0.85	X_{13}	0.99	X_7	0.85
1992	X_{13}	0.98	X_7-X_8	0.98	X_7-X_8	0.92	X_7-X_8	0.98

(1) All R^2 values are significant at P = 0.05
(2) X7: cumulative number of growth degree days
X8: cumulative number of degree days (>=25°C)
X9: cumulative number of degree days (<=25°C)
X13: summation of maximum air temperature (>=25°C)

Table 14

Results of forward multiple regression between *Fusarium* wilt incidence of 4 chickpea varieties and (G3) environmental variables [2] X1, X2, X5 and X6 collected during the experimental period 1988-1992

Variety/ Year	ILC 482		FLIP 83-46 C		ILC 3279		FLIP 84-79 C	
	Variable	$R^{2(1)}$	Variable	R^2	Variable	R^2	Variable	R^2
1988	X_1	0.89	X_5	0.80	X_5	0.85	X_5, X_6	0.98
1989	X_5	0.97	X_1	0.92	X_1	0.78	X_1	0.87
1990	X_2	0.90	X_5	0.88	X_5	0.99	X_5	0.84
1991	X_6	0.83	X_5	0.83	X_2-X_6	0.95	X_5	0.84
1992	X_5	0.80	X_5-X_2	0.98	X_5	0.92	X_5	0.96

(1) All R^2 values are significant at P = 0.05
(2) X1: cumulative number of days (>=20°C)
X2: cumulative number of days (>=25°C)
X5: cumulative number of days (max>=25°C)
X6: cumulative number of days (max<25°C)

one variety to another. However, variables X5 and X13 were involved in half the regression models and represent high temperature (Tables 13 and 14). Variable X6, maximum air temperature < 25°C, was the least frequent in the multiple regression equations (Table 14). In some varieties (ILC 3279, FLIP 84-79C) and years (1988, 1991) opposite environmental variables contributed positively and significantly to the same equations. These results are difficult to explain. Coefficients of determination (R^2) for all variables tabulated were close to or higher than 0.80.

No variable was consistently related to one variety, although X13 appeared to be frequently associated with ILC 3279 whereas X7 was frequently associated with FLIP 84-79C.

5.3.2.2. Soil temperature variables (G2 and G4)

Significant relationships between variables X3 and X4, representing the cumulative number of days with soil temperature of ≥20°C at 10 and 20 cm depth, respectively and disease progress were found in all year/variety combinations except for ILC 482 in 1991 (Tables 15 and 16). Variable X3 explained 39 to 99% of the variation in disease progression in individual years. Coefficients of determination (R^2) values were somewhat higher for varieties having a late wilting reaction, i.e. ILC 3279 and FLIP 84-79C than those that wilted earlier. Similarly, soil variables X10 and X12 representing soil degree days at 10 cm depth and soil degree days for

Table 15

Results of forward multiple regression between *Fusarium* wilt incidence of 4 chickpea varieties and (G2) environmental variables [2] X10, X11 and X12 collected during the experimental period 1988-1992

Variety/ Year	ILC 482		FLIP 83-46 C		ILC 3279		FLIP 84-79 C	
	Variable	$R^{2(1)}$	Variable	R^2	Variable	R^2	Variable	R^2
1988	X_{12}	0.92	X_{10}	0.78	X_{10}	0.84	X_{11}	0.96
1989	X_{12}	0.97	X_{12}	0.92	X_{12}	0.76	X_{12}	0.83
1990	X_{12}	0.63	X_{11}	0.88	X_{11}	0.99	X_{11}	0.90
1991	X_{11}	0.47	X_{10}	0.87	X_{10}	0.88	X_{10}	0.86
1992	X_{11}	0.78	X_{10}	0.95	X_{10}-X_{12}	0.95	X_{12}-X_{10}	0.98

(1) All R^2 values are significant at P = 0.05
(2) X10: soil degree days at 10 cm
X11: soil degree days at 20 cm
X12: soil degree days at 10 cm (>=25°C)

Table 16

Results of forward multiple regression between *Fusarium* wilt incidence of 4 chickpea varieties and (G4) environmental variables [3] X3 and X4 collected during the experimental period 1988-1992

Variety/ Year	ILC 482		FLIP 83-46 C		ILC 3279		FLIP 84-79 C	
	Variable	$R^{2(1)}$	Variable	R^2	Variable	R^2	Variable	R^2
1988	X_4	0.95	X_3	0.66	X_3	0.74	X_4	0.86
1989	X_3	0.97	X_4	0.97	X_4	0.86	X_4	0.90
1990	X_4	0.65	X_3	0.88	X_3	0.99	X_3	0.90
1991	-	-[2]	X_3	0.76	X_3	0.85	X_3	0.74
1992	X_3	0.39	X_3	0.83	X_3	0.89	X_3	0.97

(1) All R^2 values are significant at P = 0.05
(2) No significance was detected at P = 0.05
(3) X3: cumulative number of days with soil temperature >=20°C at 10 cm
X4: cumulative number of days with soil temperature >=20°C at 20 cm

temperatures >25°C at the same depth, respectively were frequently related to disease progress (Table 15). It is interesting to note that during the 1990 season, which was characterized by a severe drought, soil degree days at 20 cm depth (X11) was the main variable accounting for disease progression in three of the four varieties (Table 15).

5.4. DISCUSSION

This study was concerned mainly with the relationship between some environmental factors and the progressive development of *Fusarium* wilt of chickpea. It was done at a single location over an extended period. This has an advantage over multiple site testing as some variables such as differences in fungal pathotypes, cropping history and inoculum density in one field versus an other, were avoided. The single location approach has been used by other workers such as Pullman and Devay (1982) who used it to study the epidemiology of *Verticillium* wilt of cotton over a period of 7 years.

The chickpea varieties tested in the experiments reported in this chapter varied in the time that symptoms first appeared and disease progression. Certain varieties may be taken as reference standards for comparative purposes. For example, a variety would be considered an early wilter if its time to the onset of wilt symptoms were shorter than that of ILC 482. On the other hand it would be classified as a late wilter if the time to symptom appearance were longer than ILC 3279. As discussed in

Chapter 3, the late wilting reaction is a form of a partial resistance, governed by one or more genes (Upadhyaya *et al.*, 1983a, b). Varieties which shows this type of reaction should not be discarded by plant breeders.

In this study, high temperature (≥25°C) and cumulative number of hot days (with temperature ≥25°C) were consistently related to the development of wilt. This is in concordance with the observations made by Nene *et al.* (1979) in Northern India where he reported that wilt incidence is negligible during the vegetative stage of chickpea which occurs during the cold weather. Bhatti and Kraft (1992a) found that increasing temperature favours the development of chickpea wilt. Similarly *Fusarium* wilt of tomato grown under field and plastic greenhouse conditions was enhanced by high temperature (Besri and Zrouri, 1983). By contrast, high air temperature arrested symptom expression of *Verticillium* wilt in cotton (Garber and Presley, 1971; Pullman and Devay, 1982).

The results of this study are, probably, the first contribution to the understanding of the relationship between disease progression and field environmental variables in the FOC-chickpea system. They could have practical implications in controlling the disease. Chickpea in WANA is traditionally grown as a spring-sown crop. It is planted in March and the growth takes place in an environment characterized by increasing air temperature and by frequent terminal drought making the crop more vulnerable to wilt disease. One possibility for decreasing

disease incidence is to plant chickpea early in the season so that plant growth occurs in a cool environment. The technique of winter or early sowing of chickpea is being popularised with farmers in WANA region particularly in North Africa, with the objective of increasing yields (Saxena, 1989; Singh, 1990). As shown in chapter 4, *Fusarium* wilt development is inhibited by low temperature and chickpea planted in December may escape most of the negative effect of the disease. This will be discussed in chapter 8.

CHAPTER 6

SOURCES OF RESISTANCE TO *FUSARIUM* WILT OF CHICKPEA

6.1. INTRODUCTION

The most effective way to control plant disease is through the use of resistant varieties and wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC) is no exception. Screening for wilt resistance under both field and laboratory conditions is underway at several institutions (Nene *et al.*, 1981; Jiménez-Diaz *et al.*, 1991; Singh and Reddy, 1991; Haware *et al.*, 1992). At ICRISAT, over 13,500 chickpea accessions from 40 countries were evaluated (Haware *et al.*, 1992) and 160 accessions were found to be resistant to race 1 of FOC. Of these, 150 accessions were "desi" types which are of no commercial value in the Mediterranean areas where "kabuli" types are preferred. In Tunisia, systematic screening for resistance to FOC of "kabuli" varieties from the ICARDA world collection was started in 1989 and the results are presented in this Chapter.

6.2. MATERIALS AND METHODS

6.2.1. Plant material, planting and field screening

Chickpea varieties were acquired, planted and screened in the field as described in sections 3.2.2 and 3.2.3.

6.2.2. Characterisation of resistant chickpea accessions

The 110 chickpea varieties which were resistant (wilt incidence ≤10%) in 1989 and 1990 (section 3.3.2) were tested twice in the field to reconfirm their resistance and to increase their seed. They were planted in the Béja WSP in March 1992 in a randomized complete block design with two replications as previously described (section 3.2.3). Observations were made on the following characters using procedures described in Chickpea descriptors (Anonymous, 1985) and methods used by Pundir *et al.* (1988):

1. Days to 50% flowering (DFL): the number of days from emergence to the day on which 50% of plants have started to flower.

2. Days to maturity (DMAT): the number of days from emergence to the stage when 90% of the plants in the plot have 100% matured pods.

3. Plant height (HGT): mean height from ground level to the top of the plant (in cm.) of five randomly selected plants from each plot recorded at maturity.

4. Canopy width (CANW): average spread (in cm.) of five randomly selected plants at the maximum growth stage.

5. Primary branches per plant (PB): average number per plant of primary branches observed on five randomly selected plants.

6. Secondary branches per plant (SB): average number per plant of secondary branches of five randomly selected plants.

7. Number of seeds per pod (SPP): this was estimated by dividing the total number of seeds by the total number of pods harvested from five representative plants.

8. Seed yield per plant (SYP): seed weight obtained, after threshing, from five representative plants.

9. Hundred seed weight (HSW): after harvesting and threshing the seeds were further sun-dried and the mass (g) of 100, randomly selected, was recorded.

6.2.3. Tray-dip screening

Seedlings of resistant chickpea varieties were raised in sterilized black vertisol contained in plastic trays (30 cm X 20 cm). The trays were subdivided into cells 2.5 cm square and 4 cm deep with drain openings at their base (1 cm. diam.). One seed was planted in each cell and the trays were pressed into autoclaved riverbed sand to a depth of 5 - 7 mm contained in flat-bottom pans. Trays were watered with distilled water every 3-4 days.

The trays were lifted from the sand bed 5 - 10 days after planting and roots protruding from the cells' drain openings were rinsed with sterilized water (Fig. 16). The entire tray was placed in a shallow basin containing a spore suspension of FOC (10^6 spores/ml of isolate FG_3 prepared as described in section 2.2.5) and returned to another pan filled with an autoclaved soil mixture consisting of riverbed sand and black vertisol (1:1). The tray was left undisturbed on a bench in a greehouse until flowering. Seedlings were watered as needed with distilled

Fig. 16:

Tray-dip screening technique. Details are described in chapter 6, section 6.2.3.

water and were checked every 2 days for wilt symptoms. One to two chickpea varieties, along with a susceptible check (ILC 482), were accommodated in one tray (Fig. 16).

6.2.4. Data analysis

Data analyses included: analysis of variance, principal components analysis (PCA) based on covariance, and a cluster analysis based on Mahalanobis distance (D^2) (Mahalanobis, 1936). The purpose of PCA analysis is to reduce the number of traits measured to a small number of independent variables (called principal components, factors or axes). These are linear combinations of the original traits. The first axis (axis 1) is defined so that it explains the greatest amount of variability. The second axis (axis 2) is orthogonal to the first axis and explains the greatest amount of the remaining variability. Generally the first two to three axes explain more than 80% of the total variability. The D^2 statistic measures the divergence of populations or groups of individuals independent of the size of the sample. All data were analyzed using the statistical package STAT-ITCF (1988).

6.3. RESULTS

6.3.1. Morphological variability

Differences between varieties were significant or highly significant for all characters measured except number of days to 50% flowering (DFL), primary branches (PB) and seed yield per plant (SYP) (Table 17). Characters

Table 17

Means, ranges, standard deviations (SD) and test of significance of nine traits measured on 110 resistant chickpea varieties evaluated in spring 1992 at Béja experimental station, Tunisia.

Traits	Range [1]	Mean[1]±SD	Significance level of F test [2]
DFL	72-80	74.47±1.41	NS
DMAT	83-117	104.00±10.95	**
HGT	18-47	29.58±6.48	**
CANW	26-54	38.50±5.95	**
PB	1.60-4.30	2.96±0.48	NS
SB	1.40-6.40	3.61±1.20	*
SPP	0.70-1.50	1.02±0.14	*
SYP	1.80-17.10	6.97±3.19	NS
HSW	9.00-58.80	31.87±13.75	**

(1) *: significant at P = 0.05, **: significant at P= 0.01 and NS: non significant
(2) DFL: days to 50% flowering, DMAT: days to maturity, HGT: plant height, CANW: canopy width, PB: primary branches, SB: secondary branches, SPP: number of seeds per pod, SYP: seed yield and HSW: hundred seed weight

for which variation was highly significant (p = 0.01) were days to maturity (DMAT), plant height (HGT), canopy width (CANW) and 100-seed weight (HSW).

Correlation analysis (Table 18) showed that plant height (HGT) was highly correlated (p = 0.01) with canopy width (CANW), numbers of secondary branches per plant (SB), hundred seed weight (HSW) and seed yield per plant (SYP) and correlated (p = 0.05) with number of primary branches per plant (PB). Both plant height and canopy width were highly and negatively correlated with days to maturity (DMAT). Canopy width (CANW) was also highly correlated (p = 0.01) with number of secondary branches per plant (SB), hundred seed weight (HSW) and seed yield per plant (SYP) as well as correlated (P = 0.05) with the number of primary branches per plant. Number of secondary branches per plant (SB) was highly and negatively correlated with days to maturity (DMAT) and highly but positively correlated with number of primary branches per plant (PB). Hundred seed weight (HSW) was negatively correlated (p = 0.05) with days to flowering (DFL) and with number of primary branches per plant (PB) and highly and negatively correlated with number of seed per pod (SPP)

. In addition to the high correlation of seed yield per plant (SYP) to plant height (HGT) and canopy width (CANW), SYP was also highly correlated (p = 0.01) with days to flowering (DFL), primary branches per plant (PB) and secondary branches per plant (SB) as well as

Table 18
Correlation coefficients among some traits of *Fusarium* wilt resistant chickpea varieties

TRAIT	DFL	CANW	HGT	DMAT	BP	SB	SPP	HSW	SYP
DFL	1.000								
CANW	-0.168	1.000							
HGT	0.002	0.769**	1.000						
DMAT	0.071	-0.250**	-0.264**	1.000					
PB	0.111	0.208*	0.244*	-0.170	1.000				
SB	-0.015	0.382**	0.382**	-0.263**	0.602**	1.000			
SPP	0.235*	-0.121	-0.080	-0.024	0.056	0.070	1.000		
HSW	-0.219*	0.268**	0.310**	0.159	-0.202*	-0.106	-0.396**	1.000	
SYP	0.246**	0.454**	0.511**	-0.078	0.364**	0.482**	0.208*	0.072	1.000

(1) *: significant at P = 0.05, **: significant at P= 0.01 and NS: non significant
(2) DFL: days to 50% flowering, DMAT: days to maturity, HGT: plant height, CANW: canopy width, PB: primary branches, SB: secondary branches, SPP: number of seeds per pod, SYP: seed yield, HSW: hundred seed weight

correlated (P = 0.05) with seeds per pod (SPP).

All 110 varieties with their corresponding traits are presented in appendix 6.

6.3.2. Principal Components Analysis

Principal components analysis (PCA), based on covariance, showed that variability was well described by the first two principal components which accounted for 83.0% of the total variability (PC1 = 50.% and PC2 = 32.4%; Table 19). PC1 is mainly determined by the hundred seed weight (HSW) which had a high and positive weight and PC2 is mainly determined by the days to maturity (DMAT) trait. Apart from HSW and DMAT, all other traits had low positive or negative weights. A two dimensional representation of variability in the genetic material, using Axes 1 and 2 as coordinates is presented in Fig. 17.

6.3.3. Clustering

Cluster analysis was performed in order to partition the chickpea varieties into homogeneous groups. All varieties could be grouped into four clusters. The number and the code of varieties included in each cluster are presented in Table 20. Groups 1, 2 and 4 contain more or less the same number of varieties. The inter cluster variation, based on Mahalanobis distance (D^2), is shown in Table 21. The maximum distance was observed between clusters 1 and 4 suggesting important diversity between

Table 19

Principal Components Analysis for *Fusarium* wilt resistant chickpea varieties,coefficients of original traits for the first three principal components and % of variability.

Traits [1]	Principal components		
	1	2	3
DFL	-0.020	0.013	0.023
CANW	0.135	-0.287	0.566
HGT	0.166	-0.328	0.611
DMAT	0.211	0.890	0.400
PB	-0.006	-0.009	0.020
SB	-0.007	-0.037	0.061
SPP	-0.003	0.000	0.002
HSW	0.952	-0.096	-0.282
SYP	0.027	-0.073	0.242
Percentage of variability	50.6	32.4	12.3
Cumulative percentage	50.6	83.0	95.3

(1) DFL: days to 50% flowering, DMAT: days to maturity, HGT: plant height, CANW: canopy width, PB: primary branches, SB: secondary branches, SPP: number of seeds per pod, SYP: seed yield and HSW: hundred seed weight

Fig. 17:

Two dimensional representation of divergence of chickpea varieties resistant to FOC using the first two axes of the Principal Component Analysis as coordinates.
Varieties, identified by their code number, are presented in appendix 6.

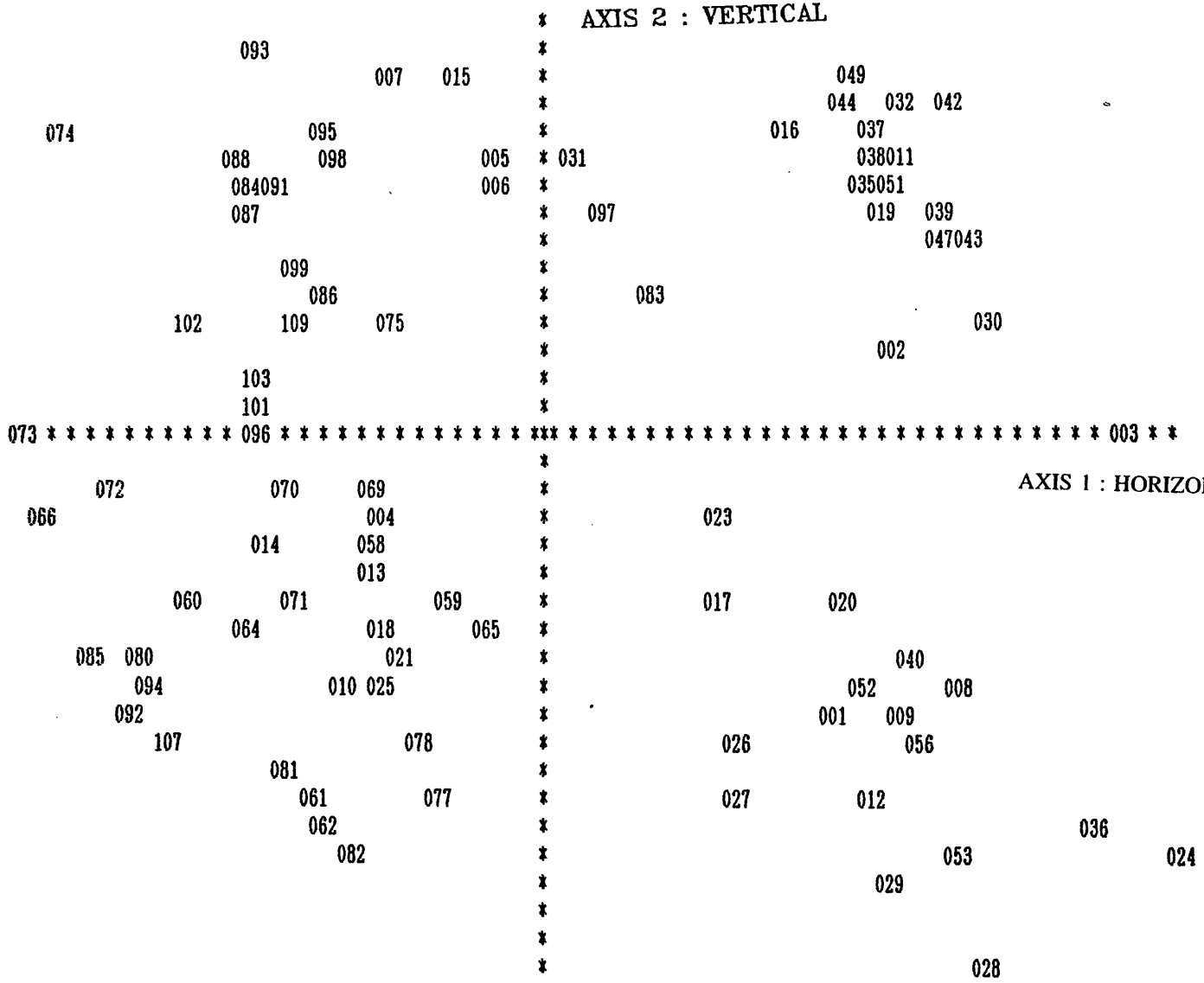

AXIS 2 : VERTICAL
093
007 015
074 095
088 098 005
084091 006
087
099
086
102 109 075
103
101
073 096 003
072 070 069
066 004
014 058
013
060 071 059
064 018 065
085 080 021
094 010 025
092
107 078
081
061 077
062
082
049
044 032 042
016 037
031 038011
035051
097 019 039
047043
083 030
002
023
017 020
040
052 008
001 009
026 056
027 012
036
053 024
029
028
AXIS 1 : HORIZONTAL

Table 20
Distribution of *Fusarium* wilt resistant chickpea varieties within each cluster along with their geographical origin.

Cluster	Number of varieties	Varieties identified by their code number	Number of varieties from		
			Mediterranean areas[1]	Asia [2]	Americas [3]
I	21	001 008 009 012 017 020 022 023 024 026 027 028 029 036 040 052 054 055 056 057	20	1	-
II	24	002 003 011 016 019 030 032 033 034 035 037 038 039 041 042 043 044 045 046 047 048 049 050 051	22	2	-
III	34	004 010 013 014 018 021 025 058 059 060 061 062 063 064 065 066 067 068 069 070 071 072 073 076 077 078 079 080 081 082 085 092 094 107	10	20	4
IV	31	005 006 007 015 031 074 075 083 084 086 087 088 089 090 091 093 095 096 097 098 099 100 101 102 103 104 105 106 108 109 110	5	2	24

(1) Spain, Tunisia.
(2) India, Pakistan, Iran, Nepal, Iraq.
(3) Mexico, USA.

Table 21

Average inter-cluster Mahalanobis distance calculated for 110 *Fusarium* wilt resistant varieties grouped in four clusters.

Clusters	1	2	3	4
1	0.000			
2	2.340	0.000		
3	2.113	2.646	0.000	
4	2.850	2.225	1.986	0.000

these 2 clusters. Minimum inter cluster distance was observed between clusters 3 and 4.

The cluster characteristics for the measured variables are presented in Table 22. All clusters had the same mean for DFL (days to 50% flowering), PB (primary branches) and for SPP (number of seeds per pod) so these characters did not contribute greatly to diversity.

Among the 110 chickpea varieties 108 were adequately grouped in the four clusters which are described as follows:

Cluster 1: High HSW (48.25 g) and early maturing (95 days for DMAT). 21 varieties.

Cluster 2: High HSW (46.84 g) and late maturing (117 days for DMAT). 24 varieties.

Cluster 3: Low HSW (22.35 g) and early maturing (93 days for DMAT). 34 varieties.

Cluster 4: Low HSW (19.62 g) and late maturing (112 days for DMAT). 31 varieties.

The average seed yield per plant was highest for varieties in cluster 1 and lowest for those in cluster 2 (Table 22).

The composition of clusters and the geographical origin of the different varieties (Singh *et al.*, 1991) are presented in Table 20. The representation of these four clusters in a plan is shown in Fig. 18.

Table 22

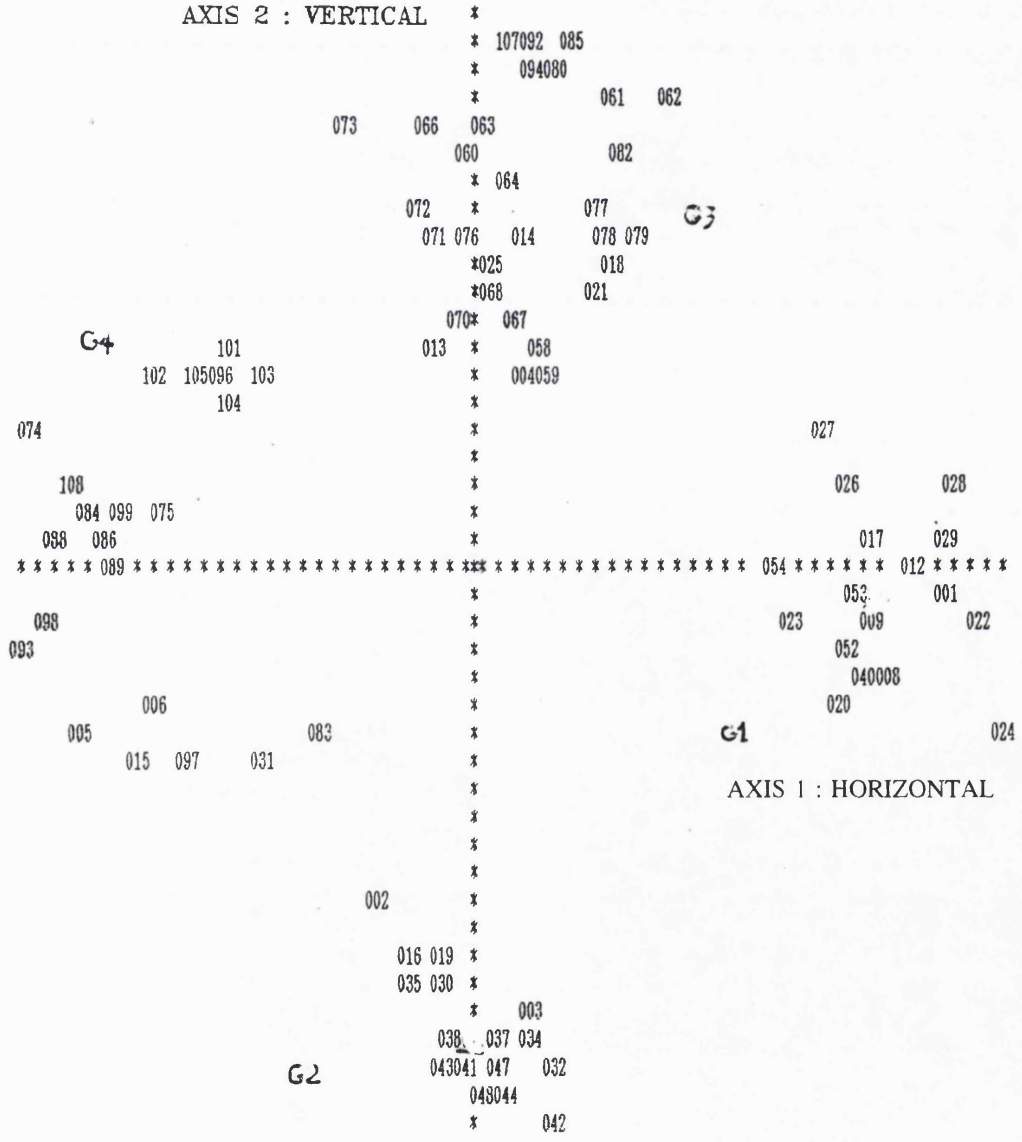
Cluster means of nine traits measured in 110 *Fusarium* wilt resistant chickpea varieties.

Trait[1]	Cluster means			
	I	II	III	IV
DFL	77.19 ± 1.40	77.12 ± 1.39	77.52 ± 1.61	77.87 ± 1.07
CANW	43.85 ± 6.18	36.04 ± 7.28	37.97 ± 4.14	37.35 ± 3.78
HGT	35.47 ± 6.63	27.83 ± 5.81	29.38 ± 5.69	27.16 ± 5.08
DMAT	95.09 ± 2.50	117.00 ± 0.00	92.97 ± 3.97	112.09 ± 4.51
PB	3.00 ± 0.46	2.72 ± 0.40	3.08 ± 0.57	2.97 ± 0.36
SB	4.22 ± 1.38	2.78 ± 0.89	3.77 ± 1.23	3.67 ± 0.84
SPP	1.00 ± 0.14	0.94 ± 0.15	1.02 ± 0.12	1.10 ± 0.11
HSW	48.25 ± 3.81	46.84 ± 2.10	22.35 ± 4.72	19.62 ± 5.37
SYP	8.86 ± 4.01	5.55 ± 2.39	6.31 ± 3.29	7.52 ± 2.05

(1) DFL: days to 50% flowering, DMAT: days to maturity, HGT: plant height, CANW: canopy width, PB: primary branches, SB: secondary branches, SPP: number of seeds per pod, SYP: seed yield and HSW: hundred seed weight

Fig. 18:

Scatter diagram of the four clusters of 110 chickpea (G1, G2, G3 and G4) varieties resistant to FOC in the plan of the first two axes of Principal Component Analysis.
Varieties are identified by their code number (appendix 6).


AXIS 2 : VERTICAL
107092 085
094080
061 062
073 066 063
060 082
064
072 077
071 076 014 078 079
025 018
068 021
070 067
013 058
004059
101
102 105096 103
104
074
027
108
026 028
084 099 075
038 086
017 029
089
054
012
053 001
098
023 009 022
093
052
040008
020
006
005 083
015 097 031
024
002
016 019
035 030
003
038 037 034
043041 047 032
048044
042
G1
G2
G3
G4
AXIS 1 : HORIZONTAL

6.3.4. Tray-dip screening

All 110 varieties were resistant when tested with this method (Fig. 19).

6.4. DISCUSSION

Several workers have identified sources of resistance to *Fusarium* wilt (Nene and Haware, 1980; Halila *et al.*, 1984; Jiménez-Diaz *et al.*, 1991; Bhatti and Kraft, 1992c). Most of these sources are of the "desi" type and very few were of the "kabuli" type. Nene *et al.* (1989) also reported several "desi" chickpea lines with broad-based and stable resistance to wilt and root rot diseases.

Recently, Haware *et al* (1992) screened over 13,500 accessions of chickpea germplasm for resistance to race 1 of FOC. They found 160 were resistant but only 10 of these were of the "kabuli" type. In this study, 1,915 were screened for resistance and 110 "kabuli" varieties were found to be resistant to the Tunisian isolate FG_3 which was shown to be race 0 (sections 2.3.3 and 3.3.2). This is the largest number of resistant "kabuli" varieties ever reported at one time.

Principal components analysis showed that there is some useful diversity in these 110 varieties. Over 50% of the diversity was accounted for by the first component to which the hundred seed weight (HSW) trait was the major contributor (Table 19). Variation in this trait has been reported by several other workers (Filippetti, 1990; Singh *et al.*, 1991; Bahl *et al.*, 1991). Varieties with *Fusarium*

Fig. 19:

Tray-dip screening technique : Resistant chickpea variety is shown in the middle of the tray with susceptible check (ILC 482) on both sides showing severe wilting.

wilt resistance and large seed size represent good parental potential in breeding programmes.

The second principal component explained 32% of the diversity and the largest contributing variable to this component was days to maturity (DMAT; Table 19). In general earliness is an important character in chickpea varieties grown under the semi-arid conditions of WANA region, particularly in North Africa where the crop is often caught in a terminal drought during reproductive growth.

All measured variables were used for cluster analysis but some did not show significant differences between varieties. In accord with the results of Jain *et al.* (1981), varieties from the same geographical region generally clustered together although there were exceptions (Table 20). For example, the most heterogeneous group was cluster 3 with 20 varieties from Asia, a centre of origin, 10 from the Mediterranean, another centre of origin (Van der Maesen, 1972) and four from Mexico. Cluster 4 was predominantly Mexican although the plant is not native to that country but was introduced there by Spaniards in c. 1500 A.D. This group also included five Mediterranean varieties and two from Asia. Clusters 1 and 2 were composed predominantly of varieties originating from Spain and Tunisia but also contained one and two varieties from Asia, respectively.

Clusters 1 and 2 are characterised by a high HSW (Table 22), an important parameter in chickpea breeding as it is a major contributor to yield (Jain *et al.*, 1981; Khan

et al., 1989; Mishra and Rao,1990; Singh *et al.*, 1990). Moreover, large-seeded chickpea varieties are preferred in Western Mediterranean countries (Halila and Harrabi, 1990; Cubero and Moreno Cubero, 1990).

The tray-dip technique was used to screen muskmelon for resistance to *Fusarium oxysporum* f. sp. *melonis* (Latin and Snell, 1986; Zink, 1992) and proved acceptable, providing that germination was uniform. In this study, the chickpea varieties germinated simultaneously and the technique proved to be an efficient method for screening them for resistance to *Fusarium* wilt. The method is simple, economical and requires a relatively small amount of space compared to field screening. It should, however, be considered as complementary to field screening rather than as a substitute.

CHAPTER 7

PHYTOTOXIC ACTIVITY OF CULTURE FILTRATES OF *FUSARIUM OXYSPORUM* F. SP. *CICERI* (FOC) TO CHICKPEA CELLS

7.1 INTRODUCTION

Many workers have investigated pathogen-produced toxins and their possible involvement in disease development (Yoder, 1980). In some instances, they have proved to be useful tools in the selection of plants that are resistant or tolerant of the pathogen that produces the toxin (Gengenbach *et al.* 1977; Earle, 1978; Daub, 1986; Vidhyasekaran *et al.* 1990). Recently, Sutherland and Pegg (1992) have reported that the race specificity of *Fusarium oxysporum* f. sp. *lycopersici* was matched by a protein fraction from race 1 which killed protoplasts of susceptible genotypes in the low μg/ml range whereas protoplasts from resistant genotypes were >100 times less sensitive.

Following these reports, it was decided to test *Fusarium oxysporum* f. sp. *ciceri* for the production of a toxin.

In one experiment isolate FG_3, determined as race 0 (see Chapter 2) was used to study the toxicity of FOC culture filtrates to chickpea cells and seedlings and, in a second experiment, both FG_3 and an isolate of race 0 from Spain were used to study the potential for using FOC

culture filtrates as means of screening for resistance to the pathogen.

7.2. MATERIALS AND METHODS

7.2.1. Chemicals and reagents

Chemicals and reagents used in all experiments were of analytical quality wherever possible. They were generally obtained from either BDH Ltd Poole, England or Sigma Chemical Co., Poole, England. Chemicals and other small experimental items supplied by other firms are indicated in the text.

7.2.2. Biological material

7.2.2.1. Fungal isolates

Two monoconidial isolates of FOC were used; FG_3 from Tunisia (classified as race 0) and race 0 from Spain. Acquisition of these isolates was described earlier (Sections 2.2.3 and 2.3.1).

7.2.2.2. Plant material

Chickpea varieties were selected from the ICARDA germplasm based on their field and laboratory reactions to FOC (Chapters 2, 3 and 6). Seeds were surface sterilized and sown in Fison's Levington compost (Fisons, Ipswich, England) in 9 cm plastic pots. The plants were raised in a greenhouse (70-80% relative humidity; 25±3°C) and watered as required. Daylight was supplemented with fluorescent light for 14 h per day.

7.2.3. Preparation of culture filtrates

Spores of fungal cultures were prepared by transferring a few sand grains from the stock monoconidial isolates onto petri plates of PDA and incubating for 8 days at 25±2°C on a bench illuminated by fluorescent light (section 2.2.2). Conidia were harvested by the addition of 5 ml sterile distilled water and gentle agitation. They were counted using a haemocytometer (Improved Neubauer model) and their concentration adjusted to 1.5×10^6/ml.

Three media were used for the preparation of culture filtrates:

1. The liquid medium (LM) of Miller and Blackwell (1986).
2. The liquid medium of Miller and Blackwell (1986) supplemented with chickpea extract (LM+CE).
3. Chickpea seed medium (CSM).

The composition of LM is presented in appendix 7. LM+CE was prepared by dissolving the constituents of LM in 1 l of chickpea extract. The chickpea extract was obtained by boiling 60 g of chickpea seeds of cultivar ILC 482 in 1 l of distilled water for 30 min and straining through 80µm nylon mesh. LM and LM+CE media were dispensed in 250 ml Erlenmeyer flasks (100 ml/flask). CSM was composed of 60 g of ILC 482 seeds. The seeds were soaked for 4 h in 60 ml of distilled water, drained and placed in 250 ml Erlenmeyer flasks.

Flasks were plugged with cotton wool, wrapped in two

layers of aluminium foil and autoclaved for 20 min at 121°C. After cooling they were inoculated with 2 ml of conidial suspension (1.5×10^6/ml). Liquid cultures were incubated at 25±2°C on a gyratory shaker (100 rpm; see section 2.2.5) and flasks with CSM were incubated on a stationary bench at 25±2°C and were hand-shaken every two to three days. Flasks inoculated with sterile distilled water and uninoculated presoaked, autoclaved seeds served as controls.

Cultures were harvested in triplicate every 7 days for 4 weeks. The fungus was removed from liquid cultures by straining through nylon mesh (80 μm) and the mycelium squeezed in order to obtain the maximum amount of filtrate. Conidia in the filtrates were counted using a haemocytometer (Improved Neubauer model) and the pH of the filtrates was recorded after further filtration under low vacuum through a glass microfibre filter (Whatman GF/A). The mycelium was recovered from the nylon mesh and dried to constant weight at 90°C. Filtrates were further clarified by centrifugation.

CSM was homogenized in 100 ml double-distilled water in a Moulinex chopper (10,000 rpm for 10 min). The homogenates were strained through six layers of cheese-cloth, clarified by centrifugation and by filtration through a glass microfibre filter (Whatman GF/A) under vacuum. After recording the pH, the culture filtrates and chickpea seed extracts were freeze-dried and the lyophilates were redissolved in H_2O to give a ten fold

concentration of the original filtrates or, for CSM the equivalent of 0.6g seed/ml.

7.2.4. Cell isolation

Leaf cells from healthy chickpea plants 10-15 days old were isolated according to the technique of Strange and Alam (1989). Leaflets (c. 20) were cut transversely into two and vacuum infiltrated with enzymes dissolved in holding buffer until most of the air spaces were filled with the solution.

The holding buffer contained the following constituents dissolved in double-distilled water to give 1 l: citric acid monohydrate, 10.5 g; $CaCl_2.2H_2O$ 5mM; K_2HPO_4 1mM; $MgSO_4.7H_2O$ 1mM; glucose 100 g (10%). The pH was adjusted to 6.1 by the addition of NaOH.

The digestion solution contained the following dissolved in holding buffer: Cellulase R10 2%; Macerozyme R10 0.3% (both from Kinki-Yakult Mfg. Co. Nishimomiya, Japan); Pectolyase Y23 0.07% (Seishin Pharmaceutical Co. Nihonbashi, Tokyo, Japan); and bovine serum albumin 0.05%. The pH was adjusted to 5.50 with 0.1M HCl.

The leaf pieces that had been vacuum infiltrated with digestion solution were stirred at about 60 rpm using a magnetic stirrer in a 10 ml beaker for 15 to 30 min until partial disintegration had occurred. Debris was removed by straining through two layers of muslin cloth and the cells pelleted by centrifugation at 85 g for approximately 5 min. The pelleted cells were washed three times in holding

buffer by alternate suspension and centrifugation before making up to 10^5 cells/ml.

7.2.5. Bioassays

7.2.5.1. Cell assay

Duplicate two fold serial dilutions of culture filtrates and extracts of CSM (section 7.2.3.) in holding buffer or holding buffer alone for controls were placed in the wells (50 µl/well) of a standard 96 well, flat bottomed microtest plate (Flow Laboratories, Irvine, Scotland) followed by cell suspension (50 µl/well; section 7.2.4.). After incubation for 3 h at 25°C, cell viability was assessed using fluorescein diacetate as a vital dye (Widholm, 1972). Fluorescein diacetate stock solution was prepared weekly (5 mg ml^{-1}) in acetone and diluted 1:50 with holding buffer before adding to each well.

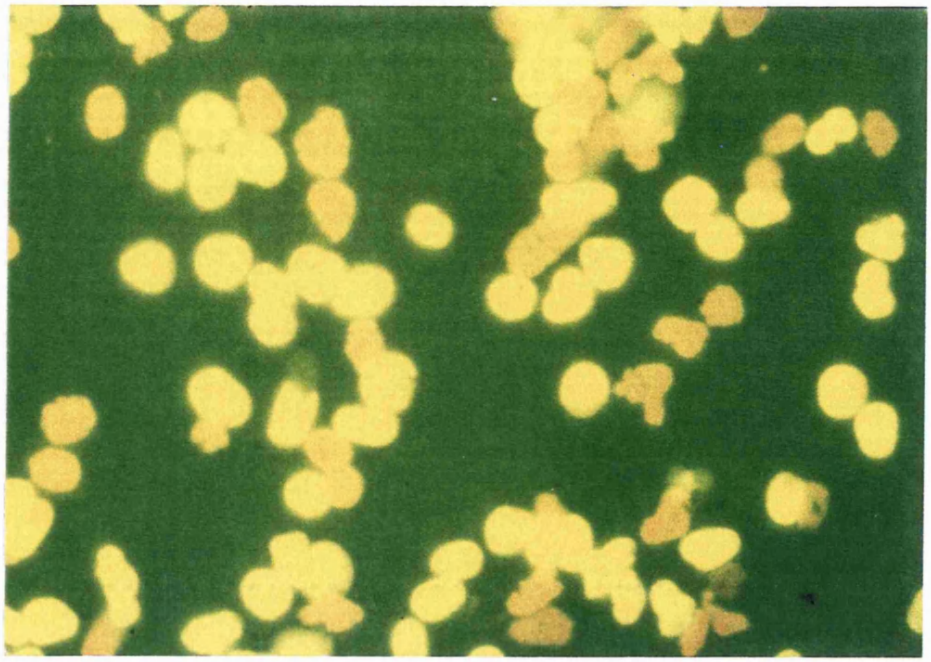
The microtest plate was viewed under an Olympus inverted microscope (Model IMT) equipped with epi-fluorescent optics. Excitation of the fluorochrome was achieved through light from a high pressure mercury burner filtered through an IF490 exciter filter and reflected onto the microtest plate by a blue dichroic mirror (DM 500) containing a built-in 0-515 barrier filter (Strange *et al.*, 1982). Under these conditions cells with intact plasma membranes fluoresced a yellow-green colour while those that were dead remained unstained. Fifty cells were counted in each well and scored live if they fluoresced and dead if they did not (Fig. 20).

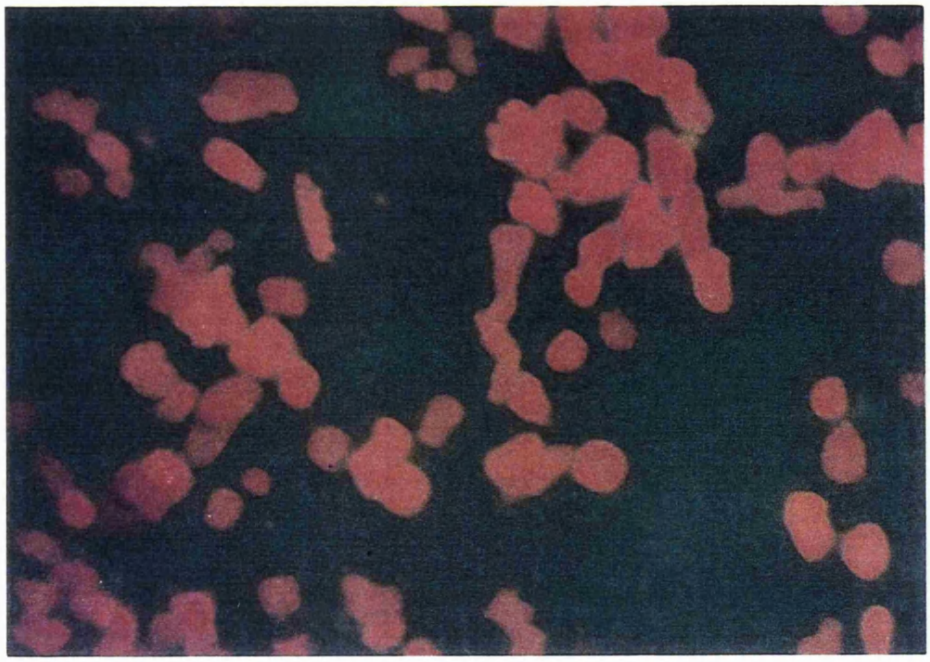
Fig. 20:

Cells of chickpea were killed when incubated in FOC culture filtrate for 3 hours.

Upper: Live cells in control wells are stained with fluorescein diacetate and fluoresce a yellow - green colour.

Lower: Cells in well containing concentrated culture filtrate. Dead cells remain unstained and show red to dark-red colour.





7.2.5.2. Seedling test

Culture filtrates from LM (15 days old) were assayed with seedlings of varieties ILC 482 and Amdoun 1 which are, respectively, susceptible and resistant to *Fusarium* wilt. Culture filtrates (50 ml) were diluted with 50 ml of sterile, distilled water and transferred to 70 ml glass tubes (200 X 24 mm diam.; 50 ml per tube) giving five replicates. Culture media, diluted 1:1 with sterile distilled water, served as controls. Chickpea seedlings (15 days old) were transferred to the tubes (two per tube). They were incubated on a laboratory bench under natural light at 20-25°C and were observed daily.

7.2.6. Expression of results

Percentage cell death was corrected for control values as follows:

$$\text{Corrected percent cell death} = \frac{C - T}{C} \times 100$$

Where C = Mean number of live cells in holding buffer only.

T = Mean number of live cells in the filtrate preparation (Strange *et al.*, 1982).

Percent cell death was converted to probit values (Appendix 8) and plotted against the $\log_2$ of the dilution factor. One unit of activity was defined as the dose

required to give a probit value of 5 (i.e. kill 50% of cells = LD_{50}). This meant converting the value on the X axis corresponding to the LD_{50} value to a linear scale to give the dilution factor and multiplying by 20 in order to ascertain the number of units per ml of toxic activity (since the volume of test solution in each well was 50 µl).

7.3. RESULTS

7.3.1. Fungal growth

Growth of the fungus was maximal on LM and LM + CM after incubation for 2 weeks (Table 23). On CSM, the mycelium covered the seed within 1 week.

The pH of culture filtrates dropped rapidly from 4.99 and 4.90 for LM and LM+CE, reaching 2.33 and 3.58, respectively, but rose later in the incubation period. The pH of the CSM homogenate was higher (7.16 - 8.40) than that of homogenate from seed that was not inoculated (6.39) throughout the incubation period.

Sporulation was greater on LM+CE than LM at all harvesting dates with the highest values in 1 week old cultures (6.30×10^6 and 24.60×10^6 spores/ml for LM and LM+CE, respectively).

7.3.2. Cell isolation

Cells were successfully isolated from all chickpea varieties with an average yield of about 3.0×10^7 cells from 1g fresh weight of leaflets). The percentage viability of cells varied from 81 to 88% in all varieties

Table 23

Mycelial dry weight, pH and sporulation obtained from FOC culture filtrates at four incubation times on three different media

CULTURE MEDIUM AND FUNGUS GROWTH	INCUBATION PERIOD (Weeks)				
	1	2	3	4	Control
Mycelial dry weight (mg/ml)					
LM	4.73±0.14	5.40±0.10	4.16±0.20	5.10±0.15	0
LM + CE	2.90±0.30	4.30±0.22	3.80±0.20	4.13±0.23	0
CSM(1)	-	-	-	-	-
pH					
LM	2.33±0.03	2.15±0.03	2.85±0.02	3.47±0.02	4.99±0.01
LM + CE	3.58±0.01	3.71±0.02	3.80±0.01	4.13±0.03	4.90±0.05
CSM	8.40±0.05	7.16±0.04	7.88±0.01	8.02±0.06	6.39±0.02
Sporulation (Nb. of conidia per ml x 10^6)					
LM	6.30±0.9	5.91±0.7	2.20±0.2	1.46±0.5	0
LM + CE	24.60±3.4	14.50±5.2	14.75±4.7	5.45±2.1	0
CSM(1)	-	-	-	-	-

(1) Not measured.

except ILC 223 and ILC 211 where it was 65 and 70%, respectively (Table 24).

7.3.3. Toxic activity

7.3.3.1. Cell assay

Cells of ILC 482 were sensitive to culture filtrates of the FG_3 isolate of FOC and extracts of the fungus grown on seed. Highest values were obtained on CSM and LM+CE at incubation periods of 2 and 4 weeks, respectively. Controls showed little or no toxicity (Fig. 21).

The cells of 10 varieties of chickpea responded similarly to extracts of both Spanish race 0 and the Tunisian isolate FG_3 grown on chickpea seed. Activity varied from 33 to 4 units/g of seed (Fig. 22). Sensitivity was related to susceptibility with the very early wilter ILC 223 being the most sensitive and the resistant varieties Amdoun1, ILC 221, UC 15 and UC 27 being the least sensitive. Other varieties were intermediate in sensitivity but this corresponded to the timing of wilting, those wilting early being more sensitive than those that wilted later (Fig. 22).

7.3.3.2. Seedling test

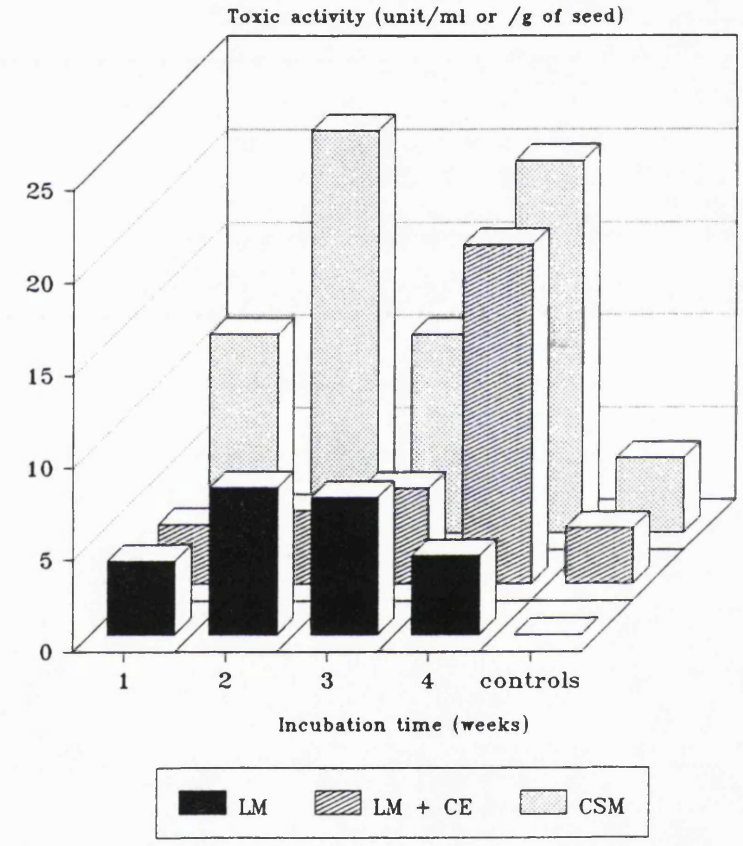
Leaflets of seedlings (variety ILC 482) became chlorotic and the seedling wilted within 48 h of incubation with a 15 day old culture filtrate of the Tunisian isolate FG_3 (Fig. 23). The chlorosis progressed

Table 24

Percentage viability of cells obtained from various chickpea varieties

Varieties	Cell viability (%)
Amdoun 1	86.70 ± 2.55
ILC 3279	85.12 ± 5.31
ILC 482	88.03 ± 3.03
ILC 233	65.30 ± 3.75
ILC 211	70.02 ± 4.30
P-2245	81.18 ± 5.14
UC 15	82.15 ± 4.99
UC 27	82.39 ± 5.79
FLIP 84-79C	83.41 ± 5.02
ILC 1929	84.70 ± 3.60

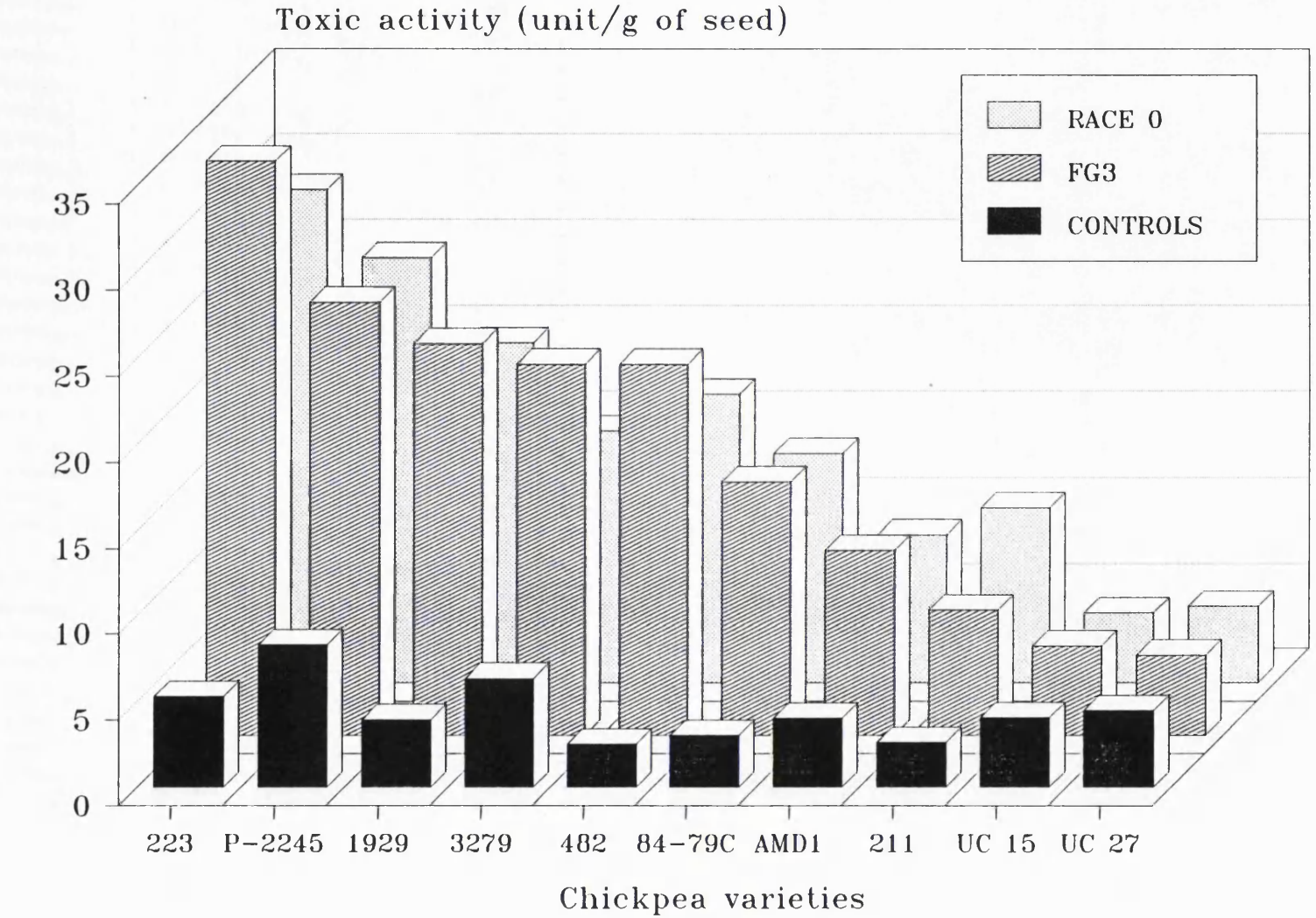
Fig. 21: Toxic activity OF culture filtrates of FOC isolate,incubated for four weeks on three media,on cells of ILC 482



(Data tabulated in appendix 9)

Fig. 22:

Toxic activity of two FOC isolates, grown on CSM, on cells of 10 chickpea varieties. Varieties are listed along the X axis in the order of their increasing field resistance to FOC (appendix 10).



(Data tabulated in appendix 10)

Fig. 23:

Chickpea seedling test with FOC culture filtrate

Upper: Development of wilt symptoms (left) 48 h after incubation of the seedling in the filtrate. Note yellowing on most leaves and collapsing of plants (left). Control plants (right) remain upright and retain their dark green colour.

Lower: Leaves (left and right) are showing progressive necrotic yellowing. Leaves from control plants (middle) are green.

rapidly upwards and leaflets sometimes curled inwards or twisted around the midvein. Seedlings of resistant variety Amdoun 1 remained green, healthy and upright as did controls (Fig. 23).

7.4. DISCUSSION

Several reports have discussed the usefulness of plant cells and protoplasts as material for the study of phytotoxins (Earle, 1978; Brettel and Ingram, 1979; Connell *et al.*, 1990). Major advantages over whole plants are their sensitivity, the requirement for only small volumes of toxin solution, speed and the absence of the complications of diffusion and translocation (Strange *et al.*, 1982). In addition, assays employing cells are more easily quantified than other techniques such as cuttings and leaf puncture assays, making for greater reproducibilty of results (Strange *et al.*, 1982; Breiman and Galun, 1981).

Where toxins are shown to play a crucial role in virulence or pathogenicity there is the possibility of using them as surrogates for the pathogen in screening procedures.

Nadel and Spiegel-Roy (1988) used a toxin produced by *Phoma tracheiphila* to select lemon cell lines resistant to mal secco, a serious disease in *Citrus*. Vidhyasekaran *et al.* (1990) screened for resistance to brown spot of rice using partially purified toxin of *Helminthosporium oryzae*. More recently, Sutherland and Pegg (1992) have reported that the race specificity of *Fusarium oxysporum* f. sp.

lycopersici was matched by a protein fraction from race 1 which killed protoplasts of susceptible genotypes in the low µg/ml range whereas protoplasts from resistant genotypes were >100 times less sensitive.

In the present study, culture filtrates of two FOC isolates, originating from two different parts of the world, showed similar toxic activity on plant cells (Fig. 22) when tested on 10 chickpea varieties which differed in their field reactions to *Fusarium* wilt. However, susceptible varieties (very early to early wilters) were most sensitive and late wilters and resistant varieties were less sensitive. This close relationship between disease reaction in the field and cell sensitivity suggest that the assay could be used as a tool in screening. The method should, however, be tested on a considerable number of cultivars with different reactions to FOC to confirm these findings and also on other races of FOC.

In the seedling test, symptoms appeared rapidly in the susceptible variety ILC 482 but not in the resistant variety Amdoun 1. The rapidity of expression of wilt symptom is quite striking. One possibility is that the cells surrounding the stelar tissue lose their turgor and therefore fail to support the plant.

The only study of biochemically characterized factors that could play a role in wilt caused by FOC is that of Pérez-Artes *et al.*, (1989). They found that FOC produced pectic enzymes and that production varied according to isolate. However, no attempt was made to relate this to

virulence.

Further studies are needed to identify and characterize the toxins.

In some instances plant extracts seem to enhance toxin production of pathogenic fungi in liquid culture. Robeson and Strobel (1986) found that *Alternaria helianthi* (Homsf.) produced toxic deoxyradicinin only if an extract of sunflower leaves was added to the medium. Furthermore, Alam (1989) demonstrated that it was necessary to add extract of chickpea seed to the medium in order to initiate and promote toxin production by *Ascochyta rabiei*. In the present experiment, CSM gave the best yield of toxic activity, whereas adding chickpea extract to LM did not enhance toxic titres significantly (Fig. 21).

CHAPTER 8

EFFECTS OF *FUSARIUM* WILT ON YIELD COMPONENTS OF CHICKPEA AS INFLUENCED BY DATE OF PLANTING AND VARIETY

8.1. INTRODUCTION

In the WANA region, chickpea is planted in the spring and harvested in early summer when it is often exposed to high temperatures and drought which may limit yield (Saxena, 1989). Not surprisingly, advancing the date of planting from spring to winter resulted in substantial increases in yield (Saxena, 1989).

Date of planting also affects the incidence of wilt. In India, for example, delayed sowing usually decreased the incidence of fungal attack but also chickpea yield (Raheja and Das, 1957; Singh and Singh, 1984). By contrast, in Spain, when planting was advanced from spring to winter, the incidence and severity of wilt was reduced in moderately susceptible varieties but not in susceptible ones (Jiménez-Diaz *et al.*, 1990b).

With the increasing unease attending the use of biocides to control plant pests and diseases, there has been a resumption of interest in control methods in which their use is limited or avoided altogether. Such methods, which often come under the term "Integrated Control", may combine many facets of crop husbandry such as the selection of resistant genotypes, crop rotation régimes, destruction of crop debris and alteration of planting date. In this

chapter the effect of planting date and variety on the incidence of wilt of chickpea caused by FOC is considered.

8.2. MATERIALS AND METHODS

In order to test the effect of planting date and variety on the incidence of wilt and its effect on yield components, varieties were planted in the 1990-1991 season in both the wilt sick plot (WSP) at Béja and in a nearby non-infested plot with a similar soil type (section 3.2.1.). The experimental design was a split plot model with three replications.

8.2.1. Planting and crop husbandry

Seedbed preparation, fertilization, planting and phytosanitary measures were performed on both plots as previously described (section 3.2.3.). Each operation was executed first in the non-infested plot and then in the WSP. Before entering the non-infested site the equipment used for cultural practices as well as wheels of tractors were disinfected in a large concrete basin containing tap water mixed with 10% sodium hypochlorite solution (4:1). After rinsing with running water the equipment was sun-dried.

Five planting dates were tested: 20 November (D1) and 15 December (D2) in 1990 and 15 January (D3), 4 February (D4) and 2 March in 1991 (D5). March is the traditional planting time for chickpea in Tunisia and other North African countries.

Four "kabuli" chickpea varieties were selected on the basis of previous experiments and observations. They were:

1. ILC 482, a susceptible variety with an "early wilt" reaction type and early flowering.
2. FLIP 87-4C, a susceptible variety with a "late" wilting reaction and early flowering.
3. ILC 3279, a susceptible variety with a "late wilting" reaction type and late flowering.
4. FLIP 84-79C, a moderately susceptible variety with a "very late" wilting reaction and late flowering.

Plots of each variety consisted of six rows, 4 m long and 50 cm apart. Two rows were used as borders, two for sampling plant materials and the remaining two for agronomic observations and evaluation of the incidence of *Fusarium*. Sixty seeds treated with benomyl (Benlate 50 WP, 3 g/kg) were planted in each row. Alleys of 1 and 2 m were kept between varieties and replicates, respectively.

8.2.2. Assessment of disease and yield components

Base stand counts of all varieties at both the WSP and the control site were made 8 to 10 days after emergence. Disease assessments were made at 7 - 10 intervals and wilt incidence was calculated as:

$$\frac{\text{Number of wilted or dead plants}}{\text{Total number of plants at stand count}} \times 100$$

Plants were considered wilted when typical wilt symptoms were observed, i.e. clear drooping of leaves with or without yellowing. Vascular discoloration was checked on plants taken from border rows and fungal isolations were attempted from diseased plants at the end of the experiment or when the variety was completely dead.

Days to 50% flowering were recorded for all varieties and treatments and, at full flowering, open-flowers were counted on three replicate samples of 10 plants, selected at random, and the average number per plant was calculated. At maturity but before harvest, 10 randomly selected plants were uprooted and the roots removed at soil level. The plants were carefully put into plastic bags and taken to the laboratory to measure the following parameters:

1. Biological yield = dry weight
2. Number of pods bearing seed
3. Number of empty pods
4. Total number of seeds
5. 100-seed weight (g) measured on three random samples per treatment.

All parameters were averaged and expressed per plant.

In order to generate values for:

1. Loss of biological yield
2. Seed loss
3. Percentage flower drop

4. Percentage flower abortion

5. Loss in 100-seed weight,

the following formula was applied:

$$\frac{\text{Values from non-infested plot - values from WSP}}{\text{Values from non-infested plot}} \times 100$$

Flower drop was calculated as the difference between total numbers of flowers counted at full flowering and total numbers of pods at maturity.

Flower abortion was calculated as follows:

$$\frac{\text{Number of empty pods}}{\text{Total number of pods}} \times 100$$

8.2.3. Data analysis

Analysis of variance (ANOVA)) was performed using the SAS statistical package (SAS, 1985) in order to partition the variation among the treatments (date of planting, variety and losses) and replicates.

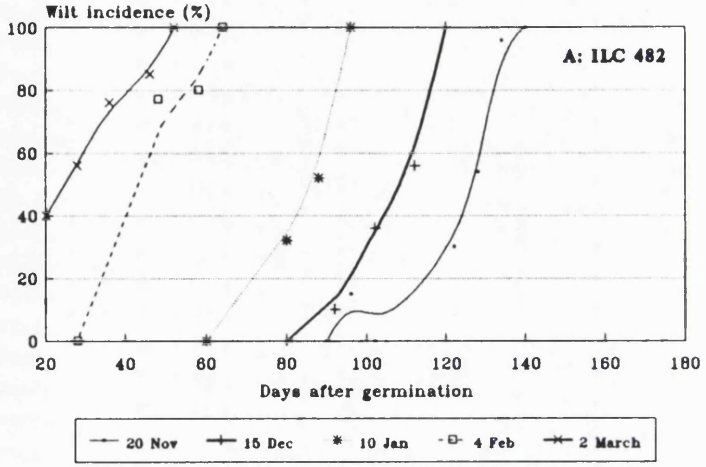
8.3. RESULTS

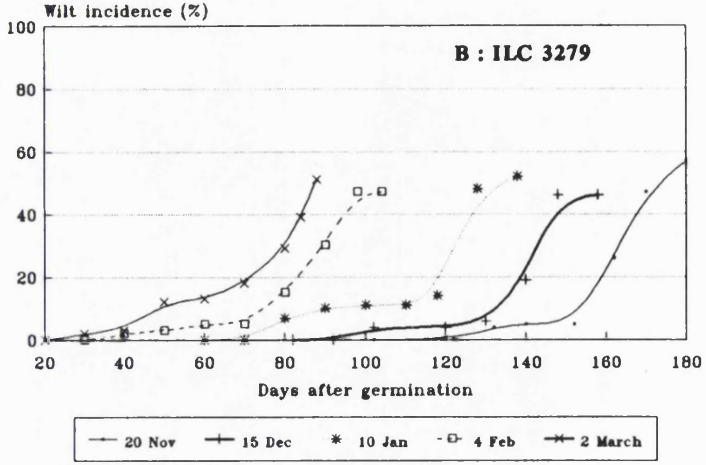
8.3.1. Disease progress

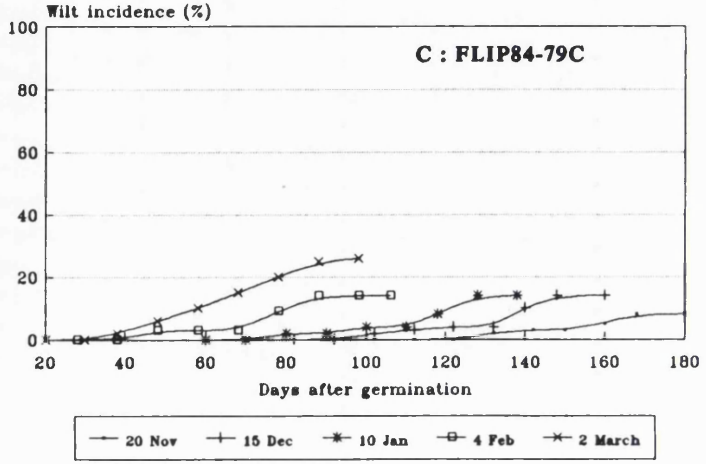
Wilt developed in the WSP but not in the nearby plot that was free of FOC. ILC 482 was the only variety to show 100% wilt and this occurred irrespective of planting date (Fig. 24A). In cultivars ILC 3279 and FLIP 84-79C the

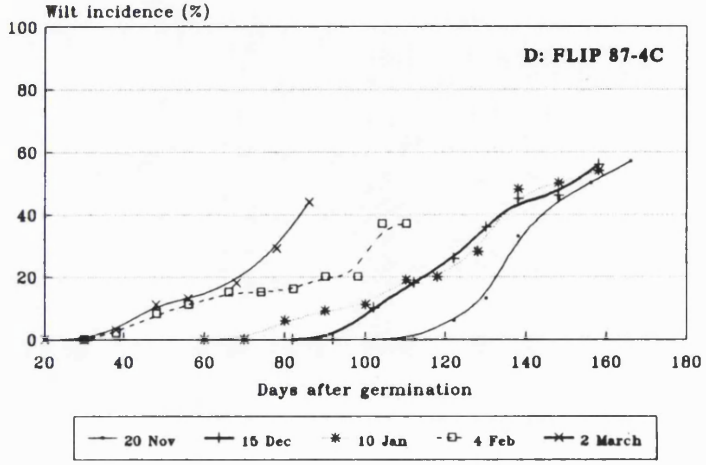
Fig. 24:

Disease progress curves for four chickpea varieties, (A) ILC 482, (B) ILC 3279, (C) FLIP 84-79C and (D) FLIP 87-4C, sown at five dates in the Béja wilt sick plot during 1990-91. Wilt incidence (%) are means of three replications.


Wilt incidence (%)
A: ILC 482
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March


Wilt incidence (%)
B : ILC 3279
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March


Wilt incidence (%)
C : FLIP84-79C
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March


Wilt incidence (%)
D: FLIP 87-4C
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March

final proportion of plants wilted was not affected very much by planting date (Fig. 24B and C). This was 40-60% for ILC 3279 but <20% for all planting dates apart from the latest (2nd March) for FLIP 84-79C. Planting date did affect slightly the final proportion of plants wilted in FLIP 87-4C; those plants sown on the three earliest dates reaching *c.* 60% wilt while those planted at the two latest dates were *c.* 40% wilted at the end of the experiment.

Planting date had a profound effect on the timing of appearance of wilt symptoms. At the earlier planting dates, wilt symptoms appeared at about 90-120 days after germination whereas with the later planting dates they appeared at < 40 days. The rate of disease progress varied also with dates of planting and varieties. It was maximum with ILC 482 and minimum with FLIP 84-79C (Fig.25A and C) and intermediate with ILC 3279 and FLIP 87-4C (Fig.25B and D).

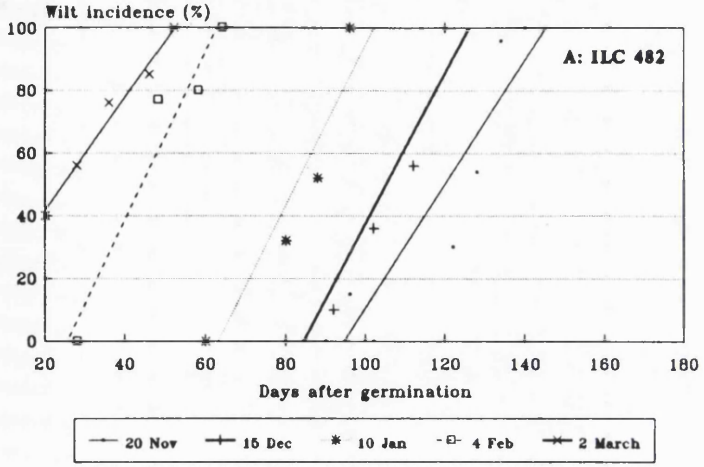
8.3.2. Effect of planting date on growth and yield components

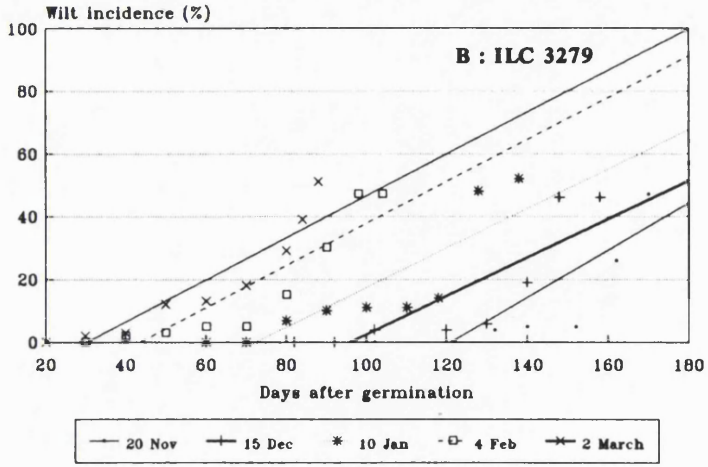
8.3.2.1. Plant growth

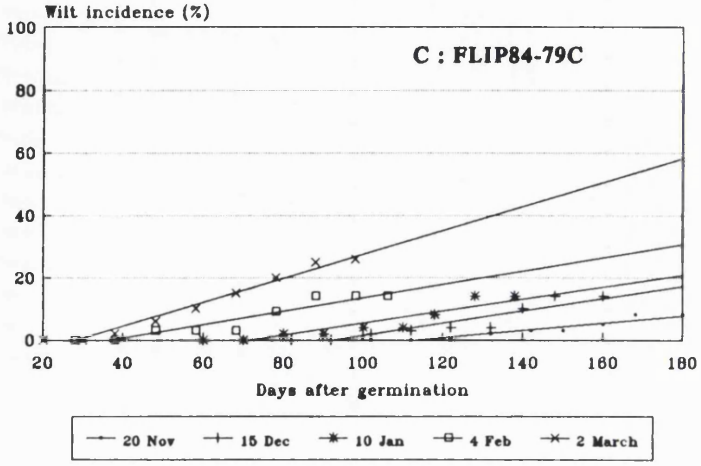
Wilt reduced biological yields of all four varieties with the heaviest losses being sustained by ILC 482 and the least by FLIP 87-4C (Fig. 26; appendices 11 and 12). Planting date did not have much effect on yield losses, the widest variation being 20.6% for variety ILC 482.

Fig. 25:

Idealised disease progress curves (Harvard Graphics Package) for four chickpea varieties, (A) ILC 482, (B) ILC 3279, (C) FLIP 84-79C and (D) FLIP 87-4C, sown at five dates in the Béja wilt sick plot during 1990-91. Wilt incidence (%) are means of three replications.


Wilt incidence (%)
A: ILC 482
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March


Wilt incidence (%)
B : ILC 3279
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March


Wilt incidence (%)
C : FLIP84-79C
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March

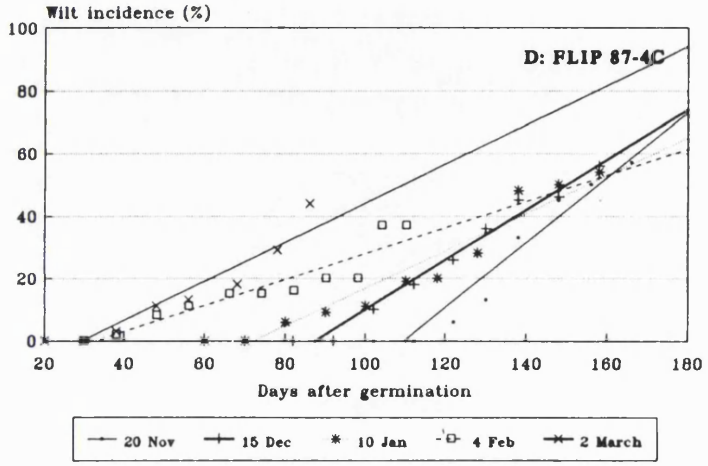

Wilt incidence (%)
D: FLIP 87-4C
Days after germination
20 Nov
15 Dec
10 Jan
4 Feb
2 March

Fig. 26:

Effect of planting dates and *Fusarium* wilt incidence on plant growth of four chickpea varieties as described by percentage loss per plant in biological yield. Trials were conducted in 1990-91 season at Béja experimental station.

D1: 20 Nov

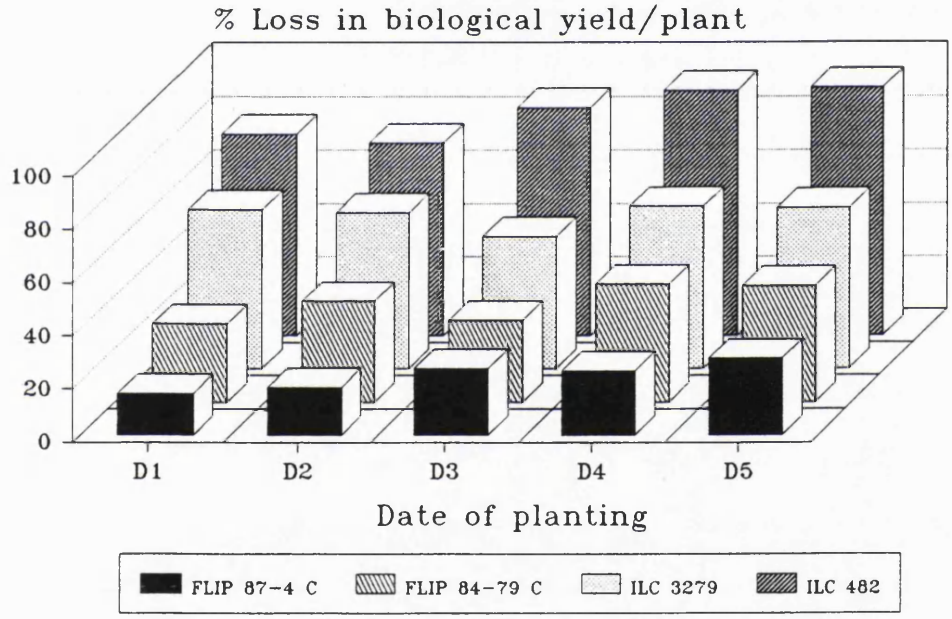
D2: 15 Dec

D3: 10 Jan

D4: 4 Feb

D5: 2 March

(Data tabulated in appendices 11 and 12).


% Loss in biological yield/plant
100
80
60
40
20
0
D1
D2
D3
D4
D5
Date of planting
FLIP 87-4 C
FLIP 84-79 C
ILC 3279
ILC 482

8.3.2.2. Yield components

Wilt had a detrimental effect on all yield parameters (Fig. 27; appendices 11 and 12). Seed loss per plant was the parameter most affected and figures for this were generally similar to those for loss in biological yield although in FLIP 84-79C and FLIP 87-4C there was a greater trend towards reduced losses at the later planting dates (Fig. 27A; appendices 11 and 12).

Flower abortion was least in FLIP 87-4C but rather similar for the other three varieties (Fig. 27B; appendices 11 and 12). There was a trend for the losses to be greater at the later planting dates and this is particularly clearly shown by the late flowering variety ILC 3279.

Flower drop was around 80% at all planting dates for ILC 482 but less than 20% at all planting dates for FLIP 87-4C and FLIP 84-79C. ILC 3279 was intermediate with about 20% flower drop at the first three planting dates but rising to about 40% and 60% at D4 and D5, respectively.

The hundred-seed weight of ILC 482 was reduced by more than 90% for the three latest planting dates, the seed being shrivelled and extremely small. However, at the earliest planting date the reduction was only 52.7% (Fig. 27D; appendices 11 and 12). Reductions in hundred-seed weight of FLIP 84-79 C and ILC 3279 were about 25% and about 50%, respectively. The hundred-seed weight of FLIP 87-4 C was slightly increased in the WSP particularly when the

Fig. 27:

Effect of planting dates and *Fusarium* wilt incidence on yield components of four chickpea varieties as described by: (A) % seed loss per plant, (B) % flower abortion, (C) % flower drop and (D) % loss in 100-seed weight. Trials were conducted in 1990-91 season at Béja experimental station.

D1: 20 Nov

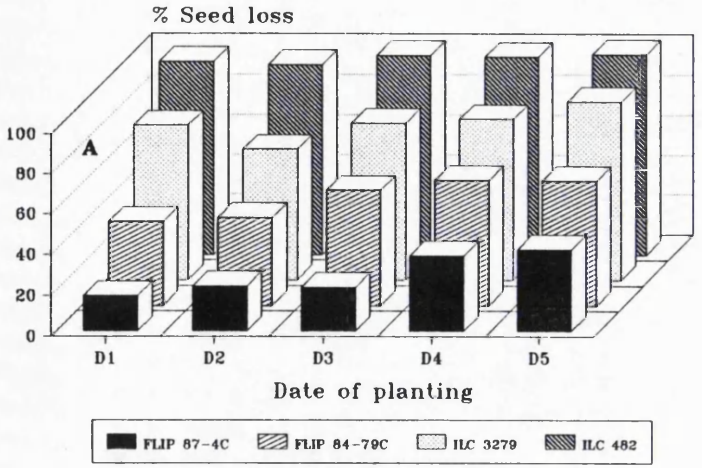
D2: 15 Dec

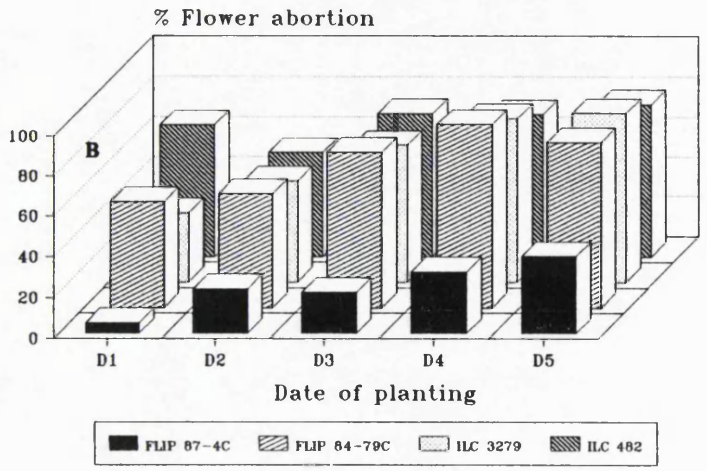
D3: 10 Jan

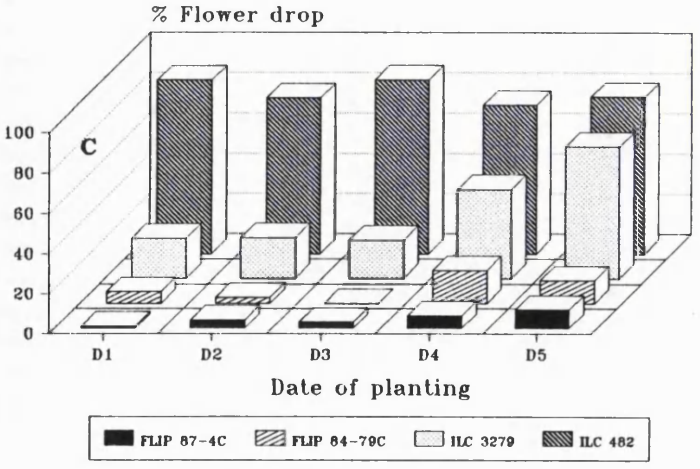
D4: 4 Feb

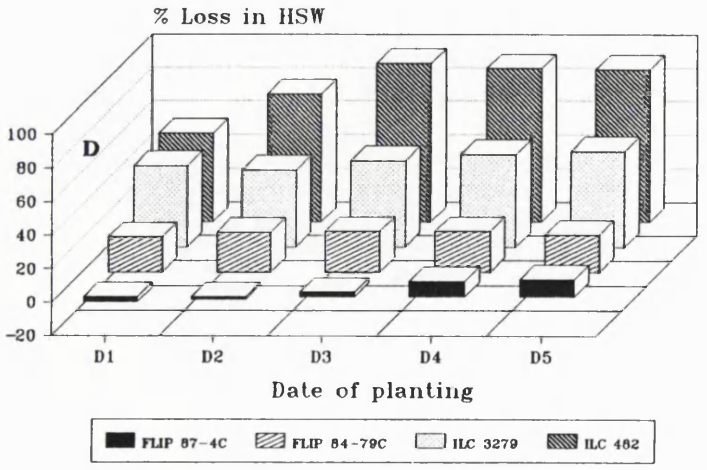
D5: 2 March

(Data tabulated in appendix 11 and 12).


% Seed loss
A
100
80
60
40
20
0
D1
D2
D3
D4
D5
Date of planting
FLIP 87-4C
FLIP 84-79C
ILC 3279
ILC 482


% Flower abortion
B
100
80
60
40
20
0
D1
D2
D3
D4
D5
Date of planting
FLIP 87-4C
FLIP 84-79C
ILC 3279
ILC 482


% Flower drop
C
100
80
60
40
20
0
D1
D2
D3
D4
D5
Date of planting
FLIP 87-4C
FLIP 84-79C
ILC 3279
ILC 482


% Loss in HSW
D
100
80
60
40
20
0
-20
D1
D2
D3
D4
D5
Date of planting
FLIP 87-4C
FLIP 84-79C
ILC 3279
ILC 482

comparison was made with the control planted at the later planting dates.

8.4. DISCUSSION

Losses, varying between 12 and 70% have been reported for wilt of chickpea (Grewal and Pal, 1970 and Jiménez-Diaz and Trapero-Casas, 1985) but these were not made by means of a direct comparison with plants grown in disease-free plots. Moreover, reports of the effect of wilt on yield of seed and other components are scarce. Haware and Nene (1980) reported seed yield reductions in "desi" varieties of 77-94%, 58-83% and 25-65% with early, medium and late onset of wilting as compared with the yields of uninfected controls. The results of this study showed similar levels of losses which were also more severe with early wilting (ILC 482) than with late wilting (FLIP 87-4 C) varieties (Fig. 27).

ILC 3279, a late wilter, suffered from substantial yield losses (65 - 89% according to date of planting) but losses in FLIP 87-4C, also a late wilter, were 17 - 40% (Fig. 27A). This variety also showed lower levels of biological yield loss, flower drop, flower abortion and reduction in 100-seed weight (Fig. 27). This result may be explained by the early initiation and expansion of open flowers, a short flowering period, speedy flower fertilization and quick pod setting followed by rapid dry matter translocation. These phenomena result in a plant that may have largely completed its reproductive cycle

before the onset of wilt symptoms. Haware and Nene (1980a), reported a similar phenomenon in the "desi" variety Chafa in which earliness rather than resistance was considered to be the reason for only moderate losses.

From the above, it appears that wilt of chickpea may be managed by arranging for the plant to reach maturity before the onset of severe symptoms. Planting varieties that mature early is one way of achieving this. Another way is to advance planting dates as observed by Jiménez-Diaz *et al.*(1990b) and Trapero-Casas and Jiménez-Diaz (1986). The effect of this is probably to allow the plant to complete its growth cycle before the temperature rises above 25°C, since symptoms are favoured by high temperatures (Fig. 28 and see Chapters 4 and 5).

Fig. 28:

Weather data measured at Béja experimental station during 1990-91 growing season.

Min.temp.:Minimum temperature

Max.temp.:Maximum temperature

Aver.temp.:Average temperature

D1, D2, D3, D4 and D5 are the following dates of planting:

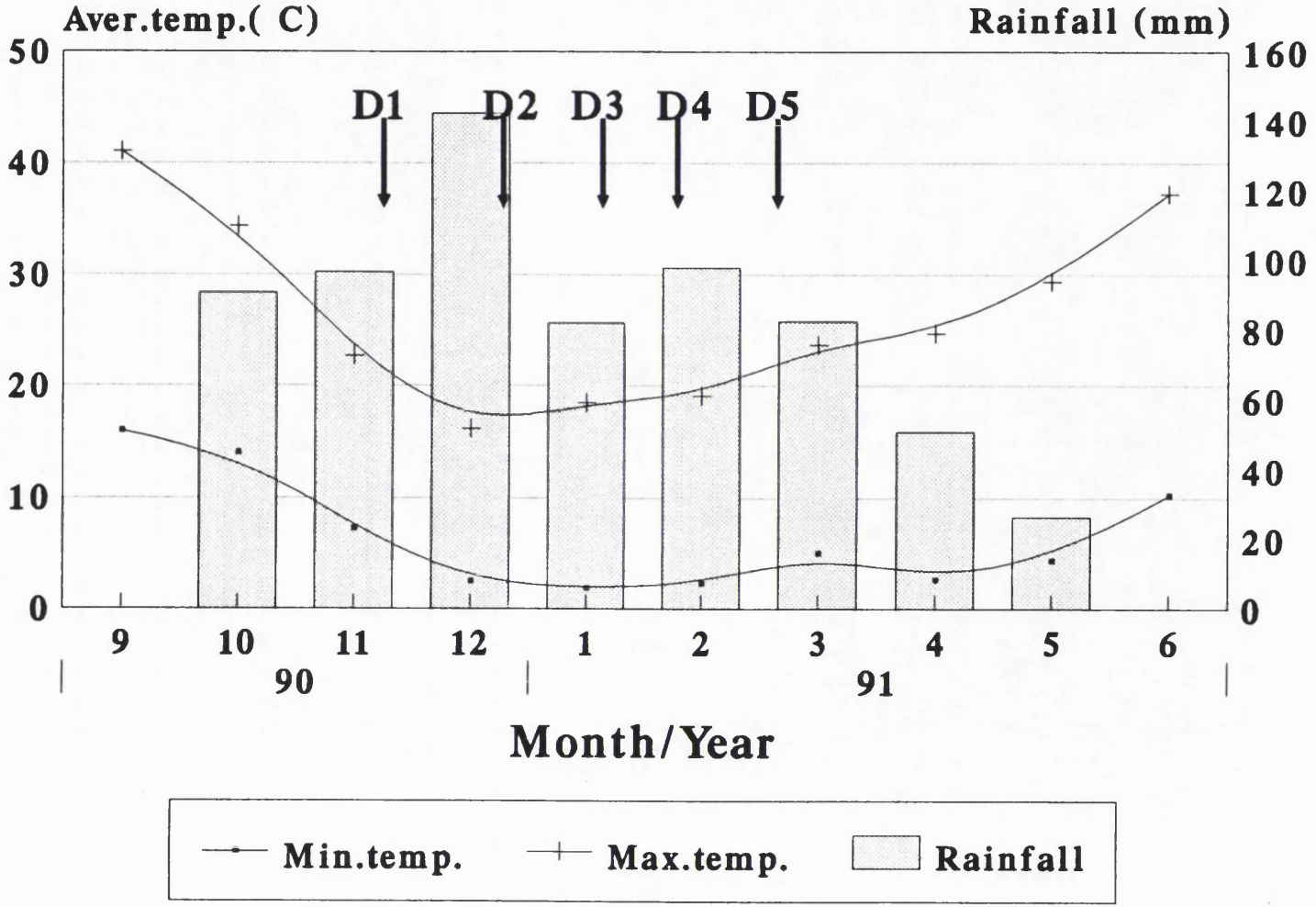
D1: 20 Nov

D2: 15 Dec

D3: 10 Jan

D4: 4 Feb

D5: 2 March


Aver.temp.(C)
Rainfall (mm)
50
40
30
20
10
0
160
140
120
100
80
60
40
20
0
D1
D2
D3
D4
D5
9
10
11
12
1
2
3
4
5
6
90
91
Month/Year
Min.temp.
Max.temp.
Rainfall

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

Studies of pathogen variability and host resistance are important in finding control measures for plant diseases. Results obtained in this study suggested that morphological variability in culture is not a valid criterion for the designation of races of FOC since isolates FG_3, F/SG_3 and FG_4, although differing morphologically, gave similar reactions on the differential varieties which defined them as race 0 (Chapter 2).

Race-specific resistance genes imply a gene-for-gene interaction. Frequently, such interactions are controlled by dominant genes for resistance on the part of the host and dominant genes for avirulence on the part of the pathogen. However, in wilt of chickpea, resistance appears to be recessive (Chapter 1). The mechanism by which this resistance is expressed is not known but in other systems in which toxins are involved, lack of a toxin receptor may provide an explanation (Strange, 1993).

Van Rheenen *et al.*, (1992) have suggested that, since resistance is race specific, durable resistance to wilt may be hard to achieve. They consider that combining resistance genes would help to stabilize resistance. The implication of these views seems to be that resistance is

dominant and that the pathogen may become virulent by mutation of the avirulence genes corresponding to the resistance genes of the host. However, since resistance is recessive in this system, it is likely to be stable since it implies the absence of an allele conferring susceptibility. This could only be provided by breeding the gene into the plant and not by mutation of an avirulence gene in the pathogen. In fact, combination of recessive genes appears to delay wilting or even give rise to resistance (Chapter 1).

The current technique for obtaining wilt resistance is to screen large numbers of lines under many wilt-endemic situations and this approach has resulted in the identification of many chickpea lines with broad-based wilt and root rot resistance (Nene *et al.*, 1989). These were "desi" types and of little commercial value in the WANA region but they could be useful as sources of resistance in "kabuli" breeding programmes.

Innovative plant breeding depends on the genetic resources available and their use. For example, results obtained in this study showed that Tunisian and Spanish chickpea varieties, selected within local landraces, provide useful sources of resistance to *Fusarium* wilt (Table 20). Similar results have been obtained with other host-pathogen systems such as barley and net blotch disease (Van Leur *et al*, 1989).

Although resistance to wilt has been identified in both "desi" and "kabuli" germplasm (Nene and Haware, 1980b;

Jiménez-Diaz *et al*, 1991; Bhatti and Kraft, 1992; Haware *et al*, 1992), the highest number of resistant "kabuli" varieties is reported in this study. Furthermore, variability in days to maturity and 100-seed weight, two important factors in chickpea breeding programmes in the Mediterranean area, was also found.

Very often "kabuli" varieties that are wilt resistant are susceptible to *Ascochyta* blight and vice-versa (Halila and Harrabi, 1990; Jiménez-Diaz and Trapero-Casas, 1990a). Combining resistance to both diseases should be an important component in chickpea improvement programmes, particularly in the Western Mediterranean areas (Halila and Harrabi, 1990; Jiménez-Diaz *et al.*, 1991).

Environment profoundly affects symptom expression in vascular wilt diseases (Nelson, 1981). Lockwood (1986) distinguished between stresses affecting pre- and post-infection events and commented on their contribution to the understanding of mechanisms and management of these diseases. Beckman (1987) further distinguished two "determinative phases" and an "expressive phase" in wilts caused by formae speciales of *Fusarium oxysporum*. The "determinative phases" were concerned with colonization and response to infection and the "expressive phase" described symptom development. He found that soil and air temperatures profoundly affected both phases and suggested that temperature should be used as a variable in studying these host-parasite interactions.

In this study, air temperatures had a direct effect

on the development of wilt. For example both the early wilter ILC 482 and the late wilter, ILC 3279, wilted more rapidly at higher rather than lower temperatures (Chapters 4 and 5).

In the WANA region, chickpea is planted in the spring and harvested in early summer when temperatures are usually well over 25°C. Advancing the date of planting from spring to winter resulted in nearly 100% gain in yield owing to more efficient use of water and the fact that growth and the reproductive phase occur in more favourable thermal and moisture conditions (Keatinge and Cooper, 1983; Saxena, 1989). Thus a late wilter, if planted in winter rather than the spring, could achieve a major part of its growth before the onset of high temperatures, resulting in avoidance or alleviation of the disease. In contrast, an early wilter, even if planted early, would become diseased resulting in considerable loss (Jiménez-Diaz *et al.*, 1990b). The early planting of varieties that were early flowering and late wilting could therefore be significant in the management of the disease.

Inoculum density should be carefully monitored in wilt-sick plots that are used for screening for resistance to FOC as too low a density might result in some varieties being scored as resistant rather than late wilters, particularly if the temperature were low. Care should also be taken with monitoring the disease since scoring too early would also bias the results towards resistance. It is better to score several times during the growth cycle

so that the different categories of wilt (i.e. early wilters, late wilters etc.) may be recognized (Chapter 3).

Screening chickpea for *Fusarium* wilt under field conditions by means of wilt sick plots is probably the most natural and efficient way to identify resistance genes which confer complete or partial (late) resistance. This selection should be complemented by screening under controlled conditions. The pot screening technique has been used successfully (Nene *et al.*, 1981) but in the present study, the technically simpler tray dip method was used and was found to give consistent results that corresponded to those obtained in the field.

Some evidence was found for toxin production by FOC (Chapter 7). Culture filtrate of the fungus killed isolated cells of chickpea and their sensitivity appeared to match the susceptibility of the varieties from which they were obtained. If this finding is substantiated and the toxin is proved to play an important role in wilt of chickpea then it may may feasible to screen varieties by means of the isolated cell technique.

In this study the toxic compound(s) in the culture filtrate of FOC was not identified. It will be interesting to purify and identify it so that it can be compared with other toxins produced by this genus. In particular, it will be interesting to know if the toxin is proteinaceous and race specific as is the toxin of *Fusarium oxysporum* f. sp. *lycopersici* (Sutherland and Pegg, 1992).

The following are topics which might be considered for

further research in wilt of chickpea caused by FOC:

1. What are the dynamics of fungal colonization of the plants belonging to the different categories of wilting (early wilters, late wilters etc.) ?

2. What is the basis of resistance or susceptibility? Are phytoalexins or toxins involved ?

3. Why is the interaction influenced so strongly by temperature ?

Appendix 1

Chickpea varieties used as differentials for race studies of FOC

Varieties	Type	Origin
JG-62	Desi	ICRISAT
WR-315	Desi	ICRISAT
JG-74	Desi	ICRISAT
Annigeri	Desi	ICRISAT
BG-212	Desi	ICRISAT
Chaffa	Desi	ICRISAT
C-104	Kabuli	ICRISAT
CPS-1	Desi	ICRISAT
L-550	Kabuli	ICRISAT
850-3127	Desi	ICRISAT
ICCV 2	Kabuli	ICRISAT
ICCV 4	Kabuli	ICRISAT
PV-24	Kabuli	Spain
P-2245	Kabuli	Spain
12-071/10054	Desi	Spain
ILC 223	Kabuli	ICARDA

Appendix 2

Papavizas's selective medium for *Fusarium* (Phytopathology 57:848-852. 1967)

	g/l
Peptone	15
Agar	20
$MgSO_4$ $7H_2O$	0.5
KH_2PO_4	1
Oxgall	1
Streptomicin	0.1
PCNB (75% WP)	1
Chlortetracycline	0.05
PH adjusted to (with 0.1M HCl)	5.2

Appendix 3

Effect of constant air temperature and inoculum levels (ID) on number of days to the onset of wilt symptoms induced by FOC in varieties ILC 223, ILC 3279 and ILC 482.

Variety and ID (cfu/g)	Air temperature (°C)				
	10	15	20	25	30
ILC 223					
4000	0	11	15	11	15
2000	0	25	15	11	12
1000	0	30	15	11	11
500	0	33	15	11	13
ILC 3279					
4000	0	31	23	13	17
2000	0	31	28	13	18
1000	0	31	28	15	19
500	0	31	28	15	19
ILC 482					
4000	0	29	16	11	11
2000	0	31	17	11	13
1000	0	31	19	12	12
500	0	31	21	12	15

Appendix 4

Number of days(1) required to reach 50% wilt incidence (t_{50}) in ILC 223, ILC 482 and ILC 3279 grown under different combinations of air temperatures and ID levels

Variety and ID (cfu/g)	Air temperature (°C)			
	15	20	25	30
ILC 223				
4000	29.1 d(2)	16.6 f	11.2 f	16.8 e
2000	30.0 d	17.0 e	11.2 f	13.1 f
1000	42.5 c	17.2 e	12.9 f	13.7 f
500	50.0 b	17.2 e	15.4 f	14.9 f
ILC 3279				
4000	49.9 b	26.8 d	15.2 f	19.0 f
2000	51.2 b	33.6 c	17.8 e	20.6 f
1000	58.9 a	38.4 c	17.1 e	21.1 f
500	65.2 a	48.9 b	20.1 e	21.0 e
ILC 482				
4000	39.9 c	21.6 e	11.8 f	13.7 f
2000	37.4 c	22.3 e	14.1 f	15.1 f
1000	40.2 c	29.8 d	13.9 f	21.3 e
500	52.1 b	32.3 c	21.8 e	16.5 f

(1) Calculated from linear regression equations **Y=AX + B**

Where **Y** = wilt incidence in % and **X**= Number of days of incubation.

- Number of observations varied with variety, temperature and ID.
- Wilt incidence = $\frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$
- One observation is a mean of three replicates (15 to 18 plants)

(2) Figures followed by the same letters are not significantly different

Appendix 5

Average height (cm) of test plants and extent of colonization (cm) of varieties ILC 223, ILC 482 and ILC 3279 grown for three weeks at 25°C in soil inoculated with FOC.

Variety and ID (cfu/g)	Height of plants[1]		Extent of colonization[1]
	Test plants	Control	
ILC 223			
4000	22.7 ± 2.1	24.6 ± 3.1	6.3 ± 1.0
1000	19.3 ± 1.9	24.6 ± 3.1	7.3 ± 0.9
ILC 482			
4000	24.0 ± 1.6	24.8 ± 2.6	16.1 ± 0.9
1000	23.7 ± 2.1	24.8 ± 2.6	15.0 ± 1.2
ILC 3279			
4000	25.3 ± 1.7	29.0 ± 4.6	16.1 ± 2.0
1000	24.5 ± 1.5	29.0 ± 3.9	17.8 ± 1.7

(1) Average of 10 plants.

Appendix 6

List of the 110 chickpea varieties resistant to Fusarium wilt with some of their agronomic characteristics

Code N°	ILC N°	CANW	HGT	DMAT	PB	SP	HSW	SYP
001	114	39	24	91	3.20	5.20	49.20	4.40
002	127	52	37	117	3.60	3.60	42.30	7.10
003	136	53	44	117	3.40	4.80	51.50	8.10
004	3107	35	24	97	2.60	2.60	27.30	4.10
005	3415	44	24	117	2.00	3.20	27.00	4.90
006	3420	40	30	117	2.60	3.80	26.50	4.70
007	3428	33	23	117	3.60	5.00	23.30	6.50
008	3437	43	34	97	2.60	5.00	51.50	10.90
009	3453	47	35	97	3.20	4.00	48.00	5.20
010	3454	40	34	93	2.80	4.60	23.50	6.10
011	3455	39	21	117	2.80	3.20	47.11	3.30
012	3457	47	34	93	2.60	2.60	48.00	4.60
013	3459	40	30	97	2.20	3.40	25.00	6.00
014	3460	34	20	93	2.70	2.90	23.50	5.50
015	3477	37	18	117	2.80	2.20	27.10	3.80
016	3480	35	22	117	2.90	2.60	42.30	3.90
017	3482	33	22	93	2.80	2.40	44.50	4.60
018	3483	38	28	93	3.20	3.80	26.80	4.20

Code N°	ILC N°	CANW	HGT	DMAT	PB	SP	HSW	SYP
019	3502	39	31	117	2.90	3.00	44.50	3.50
020	3503	35	31	97	2.80	1.80	47.80	3.90
021	3507	39	30	93	2.60	3.60	37.20	4.70
022	3514	38	31	93	3.00	2.40	50.50	3.30
023	3515	34	22	97	3.40	4.40	43.30	6.60
024	3525	52	43	97	3.80	6.40	58.80	15.20
025	3529	41	31	93	1.60	2.20	26.00	4.10
026	4098	42	35	93	3.00	4.80	42.10	7.70
027	4099	43	36	91	2.00	3.40	42.00	4.30
028	4101	52	47	91	2.60	5.80	50.50	14.10
029	4128	48	39	91	2.20	4.00	48.20	12.20
030	4130	51	32	117	3.00	2.50	47.60	7.50
031	4135	39	26	117	3.00	3.00	31.00	4.80
032	4137	27	24	117	2.60	2.80	48.70	4.30
033	4141	37	24	117	2.80	4.80	47.60	6.70
034	4143	31	29	117	3.20	1.80	46.50	5.60
035	4146	33	27	117	2.80	2.40	45.00	14.40
036	4171	50	44	97	2.80	4.00	54.90	17.10
037	4186	33	23	117	3.20	2.80	46.50	6.00
038	4194	35	26	117	3.00	2.60	45.70	5.20

Code N°	ILC N°	CANW	HGT	DMAT	PB	SP	HSW	SYP
039	4195	39	29	117	2.40	3.00	47.50	5.00
040	4198	39	35	97	2.80	2.20	49.60	8.40
041	4204	36	32	117	2.60	2.00	46.90	5.00
042	4207	28	21	117	2.00	3.40	51.00	3.80
043	4211	37	34	117	1.80	2.00	48.70	3.40
044	4213	27	25	117	2.80	3.80	45.60	6.50
045	4215	33	26	117	2.80	2.80	45.60	4.50
046	4221	37	35	117	2.40	1.40	47.70	3.10
047	4222	38	32	117	2.80	1.80	47.30	4.10
048	4230	32	28	117	2.40	2.00	47.60	3.50
049	4240	26	20	117	2.40	1.60	47.00	4.20
050	4249	28	21	117	2.60	3.80	48.00	7.20
051	4256	39	25	117	2.30	2.30	46.00	7.50
052	4265	41	36	97	3.00	3.00	46.90	7.20
053	4268	54	44	97	3.80	5.80	48.30	11.70
054	4274	54	40	97	3.20	4.80	45.50	11.80
055	4277	43	37	97	3.60	5.60	47.10	10.80
056	4284	44	39	97	3.00	4.80	48.60	12.10
057	4286	43	37	97	3.60	6.40	48.10	10.00
058	4433	38	28	97	3.00	4.80	25.50	6.70

Code N°	ILC N°	CANW	HGT	DMAT	PB	SP	HSW	SYP
059	219	41	33	97	3.00	3.20	28.10	4.20
060	267	33	31	93	2.80	3.20	17.70	4.40
061	280	43	32	88	2.90	3.20	23.50	3.70
062	299	39	26	83	2.40	1.80	26.30	3.10
063	586	37	23	91	3.00	3.60	19.50	1.80
064	588	36	26	91	2.60	5.20	21.40	2.70
065	608	38	37	97	3.40	3.20	29.40	9.40
066	3558	29	23	93	3.00	2.60	12.90	3.00
067	3596	41	34	97	3.40	3.80	25.50	7.70
068	3597	41	29	97	3.60	4.20	24.20	8.50
069	3599	33	23	97	2.80	2.00	26.90	5.60
070	3600	33	24	97	3.20	1.40	22.70	6.70
071	3601	37	34	97	3.30	4.20	20.80	11.80
072	3602	35	26	97	4.00	5.20	14.00	4.80
073	3746	31	23	97	4.00	5.60	10.70	4.20
074	3747	26	27	113	3.20	4.80	9.00	6.90
075	3748	41	36	113	3.40	4.40	20.80	10.00
076	3789	42	34	97	3.20	5.40	23.30	15.50
077	3790	46	39	93	4.30	5.70	26.30	14.20
078	3791	42	37	93	4.00	5.00	26.50	12.80

Code N°	ILC N°	CANW	HGT	DMAT	PB	SP	HSW	SYP
079	3792	42	37	93	4.20	6.20	25.60	7.70
080	3793	35	21	87	3.00	1.80	18.40	4.70
081	3400	46	42	93	2.90	3.40	18.60	7.60
082	3571	43	37	87	3.40	4.60	24.70	1.80
083	446	41	32	113	2.40	3.00	34.00	7.30
084	3714	39	23	113	3.20	3.90	16.70	7.80
085	3715	31	23	87	3.50	5.20	16.00	8.00
086	3716	38	36	113	3.20	4.60	18.00	9.50
087	3718	39	27	113	3.00	4.60	16.00	7.70
088	3719	35	23	113	2.80	3.80	16.50	10.70
089	3720	32	26	113	3.00	3.40	17.20	5.50
090	3721	36	23	113	3.00	4.50	17.80	5.40
091	3722	37	24	113	2.70	3.80	18.10	5.10
092	3723	28	25	87	2.80	4.00	17.00	4.80
093	3724	32	20	117	2.40	2.60	18.00	4.90
094	3726	35	24	87	2.40	2.50	18.10	5.90
095	3727	36	29	117	2.60	2.20	19.00	7.70
096	3728	39	35	105	2.80	4.20	17.00	7.30
097	3729	42	30	117	3.00	3.20	31.00	12.60
098	3730	40	28	117	3.40	4.60	19.00	6.50

Code N°	ILC N°	CANW	HGT	DMAT	PB	SP	HSW	SYP
099	3731	39	35	113	3.60	4.60	17.00	8.60
100	3732	38	35	105	3.00	3.20	17.00	8.00
101	3734	42	26	105	3.40	3.40	18.00	10.10
102	3735	34	24	105	2.40	1.60	16.00	7.00
103	3738	39	27	105	3.20	4.40	18.00	7.80
104	3739	32	26	105	3.20	3.60	17.00	9.00
105	3749	34	22	105	2.80	3.20	17.00	10.60
106	3741	36	22	105	3.00	3.60	16.50	6.70
107	3742	39	31	89	3.20	4.10	17.00	8.80
108	3749	40	26	113	3.00	2.80	16.00	8.10
109	3744	42	37	113	3.40	4.00	16.00	8.50
110	3801	36	22	113	3.00	4.60	17.00	9.20

Appendix 7

Liquid medium of Miller and Blackwell

(Can.J.Bot. 64:1-5. 1986)

	g/l
Peptone	2
Glucose	20
$MgSO_4$ $7H_2O$	2
KH_2PO_4	2
$FeSO_4$ $7H_2O$	0.2
Malt extract	2
Yeast extract	2
Ammonium chloride	3

Appendix 8

Table of percentages as probits extracted from Finney (1980)

%	0	1	2	3	4	5	6	7	8	9
0		2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Appendix 9

Toxic activity of culture filtrates of FOC (isolate FG_3) fungus incubated for 4 weeks in three different media against cells of ILC 482.

Incubation period (week)	Toxic activity [1]		
	LM	LM + CE	CSM
1	4.00±0.55	3.24±0.23	10.77±0.45
2	8.00±0.55	4.00±0.55	21.74±0.25
3	7.46±0.52	5.20 ± 0.89	10.77±0.46
4	4.28±0.49	18.30±0.43	20.13±0.82
Control	0	3.03±0.21	4.08±0.31

(1) Activity is expressed in unit/ml of culture filtrate or unit/g of seed (CSM). Figures are means of three replicates.

Appendix 10

Wilt disease reactions of chickpea varieties in WSPs of Cordoba (Spain) and Béja (Tunisia) compared with sensitivity of their cells to FOC culture filtrates of isolate FG_3 and race 0 grown for two weeks on CSM.

Chickpea varieties	Field Wilt reaction[1]		Nature of reaction at Béja	LD_{50} value for toxic activity of culture filtrates and controls[3]		
	Cordoba	Béja		FG_3	Spanish race 0	Controls
ILC 223	NR[2]	S	Very early	33.44±0.81	28.71±0.30	5.31±0.10
P-2245	S	S	Early	24.91±0.81	24.80±0.10	6.64±0.27
ILC 1929	S	S	Early	22.65±0.19	19.65±0.32	3.98±0.32
ILC 3279	S	S	Late	21.67±3.01	14.64±0.16	6.34±0.28
ILC 482	S	S	Early	12.99±1.89	10.11±0.10	1.53±0.13
FLIP84-79C	S	S	Very late	14.87±0.42	13.34±0.82	4.71±0.60
Amdoun1	R	R	Resistant	10.77±0.43	8.63±0.89	4.03±0.20
ILC 211	NR[2]	R	Resistant	7.25±0.17	10.22±0.20	2.63±0.13
UC 15	R	R	Resistant	5.14±0.33	5.27±0.14	4.06±0.19
UC 27	R	R	Resistant	4.63±0.77	5.01±0.14	4.43±0.30

(1) R: Resistant, S: Susceptible.
(2) NR: Not reported.
(3) Unit/g of seed

Appendix 11
Effect of planting dates and *Fusarium* wilt incidence on plant growth and yield components of four chickpea varieties

Components and dates of planting	Varieties			
	ILC 482	ILC 3279	FLIP 84-79 C	FLIP 87-4 C
% seed loss				
D_1	96.0 a	76.9 a	41.1 b	17.2 c
D_2	94.6 a	65.0 b	43.0 b	21.9 b
D_3	99.1 a	78.0 a	56.8 a	21.3 b
D_4	98.4 a	80.1 a	61.8 a	36.7 a
D_5	99.8 a	88.8 a	61.5 a	39.8 a
% flower abortion				
D_1	65.7 a	34.0 d	52.0 c	5.0 c
D_2	51.8 ab	50.0 c	56.0 c	21.7 b
D_3	71.4 a	68.0 b	77.0 b	20.0 b
D_4	71.0 a	81.5 a	91.0 a	30.0 a
D_5	76.0 a	84.0 a	82.0a	37.5 a
% flower drop				
D_1	86.1 a	20.2 c	6.0 b	1.0 c
D_2	77.1 ab	20.4 c	3.0 b	4.1 b
D_3	86.2 a	19.1 c	0.0 b	3.0 b
D_4	73.5 a	44.4 b	16.6 a	6.3 ab
D_5	77.5 a	65.6 a	11.5 a	9.2 a
% loss in 100 HSW				
D_1	52.7 c	48.4 a	21.1 a	-3.0 c
D_2	76.1 b	46.0 a	23.9 a	-1.3 c
D_3	94.6 a	51.5 a	24.5 a	2.8 b
D_4	91.4 a	55.0 a	24.9 a	9.3 a
D_5	90.9 a	57.0 a	22.5 a	10.4 a
% loss in biological yield				
D_1	75.9 b	60.0 a	29.5 b	15.7 b
D_2	72.6 b	58.8 a	38.1 b	18.0 b
D_3	85.7 a	50.0 a	30.9 b	25.1 a
D_4	92.1 a	61.3 a	44.5 a	24.1 a
D_5	93.2 a	60.8 a	43.9 a	28.8 a

Appendix 12
Effect of planting dates on
plant growth and yield components of four chickpea varieties
grown in a non infested field at Béja experimental station

Components and dates of planting	Varieties			
	ILC 482	ILC 3279	FLIP 84-79 C	FLIP 87-4 C
seeds/plant				
D_1	35.7	64.7	54.7	50.8
D_2	40.0	52.2	47.8	38.8
D_3	36.8	53.3	49.2	38.6
D_4	32.4	42.3	46.1	31.2
D_5	29.8	36.0	31.3	26.1
pods/plant				
D_1	46.8	73.0	69.3	57.9
D_2	53.1	68.3	56.4	44.0
D_3	51.3	61.3	54.0	40.1
D_4	43.8	41.8	48.3	37.2
D_5	34.9	37.8	33.2	29.7
empty pods/plant				
D_1	12.3	14.0	19.0	10.1
D_2	9.4	16.3	14.5	7.0
D_3	11.1	11.0	7.7	5.7
D_4	9.2	3.6	2.4	6.0
D_5	10.7	3.1	2.9	6.8
100 HSW				
D_1	29.3	28.6	31.6	42.9
D_2	28.2	29.1	31.4	38.3
D_3	28.6	28.2	32.2	35.7
D_4	29.3	29.3	31.0	35.9
D_5	28.4	26.0	33.2	38.7
biological yield(g) /plant				
D_1	32.9	56.2	43.6	49.4
D_2	27.8	47.5	36.7	35.0
D_3	28.6	35.0	31.7	30.7
D_4	24.3	26.1	32.0	30.7
D_5	20.3	26.8	30.6	23.2

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