AN INTEGRATED APPROACH TO PEST MANAGEMENT IN FIELD PEA, *PISUM SATIVUM* (L.), WITH EMPHASIS ON PEA APHID, *ACYRTHOSIPHON PISUM* (HARRIS)

by

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SUMMARY

This study comprises investigations into pea aphid, *Acyrthosiphon pisum*, and ascochyta blight damage on field pea, the evaluation of plant resistance levels in both breeding lines and cultivars, the identification of plant resistance and the underlying mechanisms, and cultural, chemical and biological control methods. Varietal resistance studies indicated that there were differences between the Ethiopian and the South African strains of pea aphid with regard to their survival and reproduction on the field pea genotypes evaluated. The field pea entries performed very well against the former strain compared with the latter. Three lines (Holetta Local-90, 305PS210687 and 061K-2P-2/9/2) performed well across both strains. Field pea lines exhibiting tolerance, antixenosis and antibiosis resistance to *A. pisum* were identified under greenhouse conditions. Some lines showing high levels of antibiosis to nymphal feeding were also found in both strains. This kind of resistance mechanism may promote insect biotype development through increased selection pressure on the pest population. Strain variation was also evident in tolerance, antixenosis and antibiosis resistance. The South African strain was the least aggressive across all entries. Of the 30 varieties/lines (including a local susceptible cultivar from Ethiopia) evaluated for resistance to isolates of *Mycosphearella pinodes*, Oregon Sugar Pod II had a 1.9 blight severity and was scored as resistant, three genotypes (Green Feast, Sugar Queen and line 304WA1101973) were scored as intermediate (2.1 - 3.0 severity factor) and the remaining 26 genotypes were scored as susceptible (3.1 - 4.0 severity factor) or highly susceptible (4.1 - 5.0 severity factor). In all scoring dates, significant differences occurred among genotypes, isolates and genotype x isolate interactions. However, the genotype x isolate interaction contribution to total variation was much lower than that of genotypes and isolates separately. The isolate of

the Denbi site in Ethiopia was slightly more virulent than those of the Holetta and Kulumsa sites. Assessments regarding the potential of biological control of pea aphids using a predatory beetle (*Hippodamia variegata*) and entomopathogenic fungus (*Beauveria bassiana*) indicated that predator-treated plots supported significantly lower aphid numbers from the third week onwards, when compared to the fungus-treated and infested control plots. The degree of mycosis caused by *Beauveria* on pea aphids was 14.3% in week three after inoculation and the figure dropped to 2.5% in week 5. Percentage yield loss due to pea aphid in predator-treated plots was 8.3 % compared with 16.0 % in fungus-treated plots. Field pea intercropped with Ethiopian mustard sustained less pea aphid and ascochyta blight incidence, compared to faba bean, wheat and field pea monocrop at all locations studied. The land equivalent ratio for this particular mixed crop system exceeded 1.0, indicating that the mixed crops selected were efficient for yield and monetary outcome. The increase in efficiency was ascribed to the barrier effect of mustard plants in the intercrop set-up, which was significant in reducing pea aphid population size and disease severity. The effect of fertilizer application and sowing date on pea aphid and ascochyta blight severity was location specific. At the Holetta site in Ethiopia disease severity and pea aphid infestation were significantly reduced in fertilized plots compared with unfertilized plots, while it was only the disease that showed significant difference at the Denbi and Kulumsa sites. This indicates the importance of fertilizer application as a cultural control strategy for this disease. Neither early nor late sowing resulted in reduced aphid infestation and disease infection at any of the locations. Significant interactions between variety, sowing date and fertilizer for ascochyta blight was observed, indicating that the effect of one factor was influenced by the other two factors. For aphid population density and yield, the three factors had little or no effect on

each other at the Denbi and Kulumsa sites. Cultivar Markos was moderately resistant to ascochyta blight and it gave higher yield compared to Mohanderfer and the varieties used by farmers. Neem seed kernel extract application was superior to Multineem®, a commercial product, against pea aphid development and reproduction. The neem preparations significantly reduced the number of molts, longevity and fecundity of *A. pisum* in a concentration-dependent manner. The effect on young adults exposed to neem was not as drastic as in the case of immatures. Acute and chronic toxicity effects on pea aphid were noted showing that azadirachtin is an effective inhibitor of population growth of pea aphid both on treated plants and when topically applied to the insect. Host plant resistance and natural chemical (neem) pest control in large scale farming systems, or integrated with cultural and biological control in low-input subsistence farming systems provides effective management strategies for pea aphid and ascochyta blight in field pea. From this study, possible implementation of IPM in field pea is presented and includes aspects of varietal resistance and biological, cultural and chemical control.

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CHAPTER 1

Integrated pest management of legumes with specific reference to field pea (*Pisum sativum* L.): a review

1.1 Introduction

Integrated pest management (IPM) systems were originally developed in response to insect populations with resistance to common insecticides. In many cases, the pests themselves have indicated the need for change, with pesticide resistance now a common reality in many insects, diseases and weeds. According to Kogan (1998) recognition of the development of insect pest resistance to the new organosynthetic insecticides, resurgence of primary pests, upsurges of secondary pests, and overall environmental contamination were the primary factors in the initial formulation and subsequent growing popularity of the integrated control concept. Michelbacher & Bacon (1952) were the first to use the term integrated control when describing methodologies for the selection, timing and dosage of insecticide treatments for the control of walnut aphid and preservation of beneficial arthropods in California.

Entomologists following problems related to ecological damage identified with the widespread use of insecticides in the late 1950's and early 1960's first grasped the formalized IPM concept. Wearing (1988), Allen & Rajotte (1990) and Kogan (1998) provided a detailed account of many aspects of IPM. The entomological and to some extent pathological focus are made to largely limit the scope of this review despite recognition of the valuable contributions to IPM by weed scientists.

Although there have been many attempts to seek a common definition of IPM, it is not the intent here to review in detail all these wide ranging definitions. The definition of

IPM that has been most used was coined by Smith & Reynolds (1966) during a FAO symposium on integrated pest control. They defined IPM as follows: "Integrated pest control is a pest population management system that utilizes all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury". Integrated pest control, and later IPM, had its roots in applied ecology in the 20th century, and attention to IPM was intensified in the early 1970's. The period from 1970-1988 is known as the IPM era because of the proliferation of pest management programs that adopted the IPM philosophy, both in the private and public sectors (Rajotte, Kazmierczak, Norton, Lambur & Allen 1987).

There is no doubt that an integrated control approach is the only management strategy for present and the future control of pests. During the past 20-30 years, interest in entomological biological control and IPM has increased largely as the result of problems associated with extensive use of chemical pesticides. Likewise, research and development of fungi as mycopesticides have gradually increased throughout the world.

CAB International assessed IPM and the environment in 2000 (Altieri 1987), and several papers and reports attempted to project IPM towards the next century (*e.g.* Vinson & Metcalf 1991, NRC 1996). Kogan (1998) noted that the excitement about genetic engineering, however, dominates the futurist literature in IPM. If there is a lesson to learn from the past 35 years, it is that a silver bullet is unlikely to come out of the new technologies, and nothing would have been learned from the past if genetic engineering were emphasized over all other technologies that are also blossoming. New races or biotypes of pests are developing that overcome host plant resistance (both transgenic and nontransgenic). For example, over 150 fungal or bacterial species, 500 arthropod species and nearly 270

weed species are reported to be resistant to one or other synthetic chemical pesticide (Benbrook, Groth, Holloran, Hansen & Marquardi 1996). More widespread use of host plants with transgenic resistance will likely encounter similar problems. New biologically based pest control products are likely to increase in importance because of pest resistance problems. Any IPM program includes basic components that are indispensable for its development and implementation, whether explicitly in its organization or not. Description of these components has been the object of general reviews (van den Bosch & Stern 1962, Geiter 1966).

Van Emden (1965) first suggested an interesting possibility of using partial plant resistance in combination with natural enemies, which might give economic levels of control for some agricultural insect pests. Since then, a considerable body of work has accumulated regarding mechanisms that support this notion. Van Emden (1986) provides a useful account of plant resistance - natural enemy interactions associated with microphagous (sucking insect) herbivores. Gowling & van Emden (1994) showed this for *Metopolophium dirhodum* (Walker) and the parasitoid *Aphidius rhopalosiphi* De Stefani Perez, on particularly resistant and susceptible cultivars of wheat in the glasshouse, as well as for *Brevicoryne brassicae* L. on brussels sprouts in the field, where hoverflies were the main predatory group. Van Emden also cites a number of studies showing that partial plant resistance or environmental variables (*e.g.* reduced application of nitrogen fertilizer to plants) can not only reduce aphid size and fecundity, but may also substantially reduce the weight and fecundity of female parasitoids (*A. rhopalosiphi*) emerging from the aphids. Such studies of specific systems are essential if plant resistance and biological control are to be combined in a compatible manner in pest management programmes.

Changes in control tactics have been substantial, and among those most likely to impact IPM are: i) the development of selective pesticides and botanicals (Hodgson & Kuhr 1990, Prakash & Rao 1997), ii) application of genetic engineering to the development and release of pest-resistant crop cultivars and natural enemies of arthropod pests (Meeusen & Warren 1989, Lal & Lal 1990, Hruska & Pavon 1997), iii) advances in semiochemical identification, formulation, and practical applications (Carde & Minks 1995, Howse, Stevens & Jones 1995) and iv) advances in trap cropping and in habitat management to enhance natural enemies.

The classical integrated control programs for apple orchard pests in Nova Scotia, Canada (Pickett, Putman & Roux 1958) and for cotton pests in Peru (Dout & Smith 1971) provides some of the only models for successful implementation of IPM in the field. One of the best IPM success stories is that of the Campbell Soup Company's IPM implementation with grower suppliers in USA. In 1989, this Corporation made IPM implementation a priority and in 1994, pesticide use by their growers had been reduced by approximately 50% with no loss of yield or quality (Jacobsen 1997). According to the same author similar IPM success stories have been documented for potatoes, cotton, sweet corn, soybean and most fruit and nut crops.

It must be stressed that advocating IPM does not imply outright condemnation of pesticide use. Indeed, it can still be used within the context of IPM, although such use demands more careful analysis. This should emphasize the importance of realistic economic injury levels to determine the need for control action, protect and preserve naturally-occurring biotic mortality agents (predators, parasitoids and pathogens), and apply selective chemical pesticides only when necessary and when their use is economically and

ecologically justified. For example, in an integrated pest management program targeting a glasshouse whitefly experiment, conventional insecticides were used at one-third rates in conjunction with a mycoinsecticide (*Beauveria bassiana*: Bovarol) and the parasitoid *Encarsia formosa* Gahan (Dirlbek, Dirlbekova & Jedlica 1992). The selection of appropriate insecticides (at reduced rate), careful timing and integration of all three control methods gave optimum whitefly control on certain lines where one method alone was found to be inadequate.

1.2 Field Pea (*Pisum sativum* L.)

Fourteen species of grain legumes are extensively cultivated for human consumption in different parts of the world, one of which is the field pea, *Pisum sativum* L. In many tropical developing countries of the world, a number of grain legumes are cultivated and form a high proportion of plant protein in the human diet. Peas are grown as a crop the world over but, due to sensitivity to extremes of climate, are largely confined to temperate regions, and the higher altitudes or cooler seasons of warmer regions. Pea production is restricted in the Transvaal area of South Africa because of frost during flowering period (Gane 1985). Canada, France and China account for most of the estimated world annual production.

In my own country, *viz.* Ethiopia, Assefa (1980) listed 12 species of grain legumes grown in low altitude (< 1500 m a.s.l.) areas, while Ohlander (1980) reported 28 species, including highland pulses, grown in the country at one time or another. Of the different grain legumes grown in the highlands of Ethiopia, field pea (= dry pea) is widely accepted after faba bean, *Vicia faba* L., followed by chickpea, *Cicer arietinum* L., lentils, *Lens culinaris* Medikus, and grasspea, *Lathyrus sativus* L.

Pulses are the second most important crops after cereals. Highland pulses cover 86% of the pulse area and provide 88% of the total pulse production. The highland pulses, also known as 'cool season food legumes' are essentially temperate or sub-tropical crops. Ethiopia is a secondary center of diversity for highland pulses. Faba bean and field pea grow during the main rainy season, June to October, while chickpea, lentil and grasspea grow on residual moisture (August to December). These crops are also grown on small scale during the small rains in the off-seasons, March to June, on extreme highlands (> 2500 m).

Faba bean and field pea grow, either singly or in mixture with each other, between altitudes of about 1800 and 3000 m above sea level with annual rainfall of 700-1000 mm. Those grown between altitudes of about 1800 and 2200 m are considered mid-altitude crops and those grown between 2200 to 3000 m are high altitude crops.

There are now only two species recognized in *Pisum, i.e.* the cultivated pea, *P. sativum* L., and the eastern Mediterranean *P. fulvum* Sibth and Smith (Kupicha 1981). Gentry (1971) recognized six subspecies: *abyssinicum*, *jomardi*, *syriacum*, *elatius*, *arvense* and *hortense*, lamenting that few germplasm collections have been assembled in centers of origin or diversity. Field peas grown in Ethiopia are mainly two types, *arvense* and *abyssinicum*. The crop requires a cool, relatively humid climate and is grown at higher altitudes in tropics with temperatures from 7^0-24^0C, with optimum yields between 13^0 and 21^0C (Duke 1981).

1.2.1 Origin

Field pea belongs to the same species, *Pisum sativum* L., as the more common garden variety of pea. The field pea is an early flowering, widely adapted plant. Peas are one of the oldest domesticated crops, with archaeological evidence showing that peas were brought

under cultivation with wheat, barley millet and other crops in the stone age, more than 20,000 years ago (Pritchard 1993).

Peas are native to the mountainous regions of South-West Asia and Vavilov (1949) describes these three centers as places where peas are originated, I. Afghanistan and India, II. Transcaucasia (the Asia center) and III. Ethiopia (Abyssinian center). The Mediterranean region is only a secondary center of origin. The garden pea is not found in the wild, but the field pea is found wild in hedges, cultivated ground, forests and mountainous districts throughout Europe and Western Asia (Pritchard 1993). At present it is grown throughout the world. Peas like chickpea, lentils and faba beans, are crops of which the evolution is associated with the rise of civilizations in the eastern Mediterranean and the Fertile Crescent (Buddenhagen 1990).

1.2.2. Distribution

Field pea, which is a popular vegetable and pulse crop, can be cultivated wherever cool temperate conditions prevail, *i.e.* as a summer crop in northern Europe or winter crop in the south, as a cool-season crop in the semi-arid tropics, or a year round crop at high elevations in the tropics.

Peas require a cool, relatively humid climate and are gown at higher altitudes in tropics with temperatures from 7^0-24^0C. Temperatures above 27^0C shorten the growing period and adversely affect pollination. Peas can be grown successfully during mid-summer and early fall in those areas having relatively low temperatures and a good rainfall, or where irrigation is practiced (Duke 1981). The climate in Ethiopia varies from tropical to semi-desert, desert and a permanently humid climate with a hot summer. However, the most important legume crops are grown in the highland regions, at altitudes of between 1800-2400

m where the annual rainfall varies from 950-1500 mm. These include faba bean, field pea, chickpea and lentils. Other relatively newly introduced species such as soybean, cowpeas, lima beans and haricot beans are also grown on small scale and predominantly at lower altitudes.

The crop is cultivated in all regions in Ethiopia. However, the greatest concentrations of cultivation areas are Shewa (central part), Arsi- Bale (southeastern), Wolo and Tigray (north) and Gonder and Gojam (northwest). Moreover production of the small cereals in the highlands of the country must be rotated with one of the highland food legumes to improve soil structure by fixing atmospheric nitrogen.

1.2.3 Production

Archaeological evidence indicates that peas were cultivated in Neolithic times, but, although among the first crops to be exploited by early man, it was not until Tudor times that the garden pea was first cultivated for use as a fresh vegetable (Genders 1972). *Pisum sativum* ranks second among the world's most important grain legume crops, where France, Canada and China are the largest producers (Russia was not in the table)) (Table 1.1). In 1998 a total of 12.6 million metric tonnes were produced from 6.4 million ha in more than 25 countries worldwide (Table1.1). According to the FAO Yearbook (1999), the production of field pea (dry pea) in France was 3200 thousand metric tonnes, followed by Canada (1762 thousand metric tonnes) and China (1250 thousand metric tonnes) while that of Ethiopia in the same year was 160 thousand metric tonnes and this makes Ethiopia the largest producer in Africa and 9th in the world. Africa accounts for 2.7% of the total area and production of field pea in the world. In 1999/00 Ethiopia's production of all major crops from private peasant holdings was 8,890,996 tonnes and of these pulses were 959,449 tonnes (10.8%).

The area cultivated under field pea in Ethiopia was 152,200 ha in the same year (CSA 2000). This did not include 'belg' (small rain) season production. Subsistence farmers whose yields average amongst the lowest in the world (Table 1.1 & 1.2) produce the bulk of Ethiopian grain crops. Farmers' yields of these crops are very low, ranging between 0.6 and 1.1 t/ha, potential yields under farmers' conditions can range from 1.2 to 4.5 t/ha. This wide gap between actual and potential yields is due to major production constraints and insufficient dissemination of improved technology to the farmers.

1.2.4 Usage

Peas have been the stable diet of man and livestock since prehistoric times, and are cultivated for fresh green seeds, tender green pods, dried seeds and foliage. Green peas are eaten cooked as a vegetable, and are marketed fresh, canned, or frozen-ripe. Dried peas are used whole, split or made into flour, and eaten by humans and livestock. Leaves are used as a pot-herb in Burma and parts of Africa (Duke 1981). Their high protein concentration makes them a valuable, yet cheap substitute for meat and other high-protein animal products in developing countries.

Food legumes are the major source of protein and an important component of farming systems in sub-saharan Africa, providing a major part of the daily diet of the population. Because of their high protein content (23-40%), food legumes provide a major portion of the daily protein requirement, thus alleviating malnutrition problems in the country. Their ability to fix atmospheric nitrogen, and to improve soil structure in the cereal-dominated cropping systems are key to the systems' sustainability (Osman, Ibrahim & Jones 1990). The amount of nitrogen symbiotically fixed by food legumes may exceed 100 kg/ha/year (Saxena 1988).

Farmers recognize and appreciate the ability of pea to "replenish" the soil when planted after cereal crops, and have been practicing crop rotation in Ethiopia for many years (Mamo & Dibabe 1994).

In Ethiopia, field pea plays an essential role in the nutrition of the population by balancing the deficiencies of a basically cereal diet, especially to the people of the predominantly rural areas of the country. Although the livestock population is reported to outnumber its owners, animal protein is somewhat of a rarity in the human diet. Ethiopians consume food legume products in general prepared in one form or another every day. Seeds are eaten either green or dry seeds are cooked, boiled or roasted and powdered seeds are used for making sauce. Crop residues of these legumes provide an important livestock feed. Yetneberk & Wondimu (1994) reported that about 13 traditional food types are prepared from legumes including field peas, and consumed in various ways. It is a good source of protein (20-40%), which is approximately three times that of cereals. It also contains carbohydrate (60%) and is a fairly good source of thiamin, niacin, calcium and iron (Aykroyd & Doughty 1977). Dried peas contain 10.9% water, 22.9% protein, 1.4% fat, and 60.7% carbohydrate (Duke 1981).

1.3 Insect Pests and Diseases

The pest spectrum on legumes is large and extremely diverse. Legumes are among the most heavily attacked crops and the published list by Singh, van Emden & Ajibola (1978) comprises more than 500 pests. Across the world, over two-dozen insect pests attack peas during all growth stages. Only a few have been shown to be important on a global scale. The most important insects affecting field peas are leafminers, thrips, and numerous aphid species. According to Davies, Berry, Heath & Dawkins (1985), the most common pest of field peas in Europe are pea aphid (*Acyrthosiphon pisum*), pea moth (*Laspeyresia nigricana*),

pea leaf weevil (*Sitona lineatus*), pea midge (*Contaniria pisi*); pea weevil (*Bruchus pisorum*) (Mrowczynski & Sobkowiak 1998). However, Hardie & Clement (2001) indicated that the pea weevil is the most important pest of cultivated pea in Europe, India, North and South America and Australia.

Twenty species of insect pests have been recorded on highland pulses under field and storage conditions in Ethiopia (Gentry 1965, Hill 1966, Schumutterer 1969 and 1971, Crowe Gebremedhin & Abate 1977, Abate, Gebremedhin & Ali 1982). Of these the major ones are *Helicoverpa armigera* (Hübner), *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch, *Aphis fabae* Scopoli and *Agrotis segetum* (Denis et Schiffermuller). In Ethiopia, about a dozen species attack all parts of field pea plants at every stage of growth, as well as seeds in storage (Ali 1986, Ali & Habtewold 1994). The most common and destructive pests are *H. armigera* and *A. pisum*. The bean bruchids, *Callasobruchus chinensis* (L.) and *C. maculatus* (F.) constitute the major pests of stored seeds.

Field pea is grown under a wide variety of soil and climatic conditions and numerous diseases have been reported on the crop. These include fungi, bacteria, viruses and nematodes. Twenty diffrentt pathogens have been recorded on field pea in the world (Hagedorn 1985). Fungal pathogens like downy mildew, *Peronospora viciae*; leaf and pod spot, *Ascochyta pisi,* and gray mold, *Botrytis cinerea*, are the most important foliar diseases in Europe. Powdery mildew, *Erysiphe pisi*, is a major problem in dry regions such as southern Europe and India. Soil-borne pathogens that affect root development cause large yield losses. Ascochyta blight, powdery and downy mildews, septoria blotch, damping-off and *Fusarium* root rot are the most serious fungal diseases in Australia (Barbetti & Brown 1993). Gorfu & Beshir (1994) reported fifteen fungal diseases affecting field pea in Ethiopia.

Of these diseases, ascochyta blight and powdery mildew are the most widespread and destructive. According to these authors, ascochyta blight is primarily caused by *Mycosphaerella pinodes* (Berk & Bloxam.) and to a lesser extent by *Ascochyta pisi* Lib. Both are a serious economic threat to *Pisum sativum* L. in Ethiopia.

1.3.1 Pea Aphid, *Acyrthosiphon pisum*

Aphids are undoubtedly the most important pest insects in the agriculture of the temperate climatic zones. About 4000 species of aphids have so far been described, mostly from the temperate regions of the world (Dixon 1987). Their distribution is thought to reflect their greater ability to survive the physical conditions that prevail in temperate regions (Bodenheimer & Swirski 1957). However, aphid pests of crops have often retained their pest status when introduced from the temperate into tropical and subtropical regions of the world. According to Dixon (1987), species from all aphid subfamilies live in the tropics and subtropics. There are also holocyclic endemic species in the tropics and subtropics, well adapted to the climatic conditions that prevail there.

There have been many serious outbreaks of the pea aphid, since its first appearance in 1877 in the USA (Glover & Stanford 1966) and in Australia and New Zealand (Cameron & Walker 1989). In North America, the first damaging populations of pea aphid were observed in the late 1800's. By 1900 it had spread from the eastern seaboard to Wisconsin, and by 1926 to the Pacific Coast and into Canada and Mexico (Campbell 1926). Alfalfa and canning peas are the crop most harmed in North America. Most attempts to introduce natural enemies against pea aphid have been in North America, and recently in South America since these serious pests have invaded the New World without their native parasitoids. Outbreaks can occur at regular intervals without any predictable pattern, dependent upon seasonal

conditions. In California's Mediterranean climate, the pea aphids reproduce asexually throughout the year on various legumes and the pest outbreak usually occurs in April - May or September - October (Pickering & Gutierrez 1991).

Aphids cause extensive crop losses as direct pests and as vectors of plant viruses, including bean yellow mosaic virus, red clover vein mosaic virus and pea streak virus (Barnett & Diachun 1986). Aphid feeding reduces both weight and caloric content of young pea plants by as much as 64 and 113% respectively, over 11 days, depending on the number of feeding aphids (Barlow, Randolph & Randolph 1977). They tap photosynthate, an energy source of the plant. Loss of photosynthate by aphids can have drastic effects on plant growth and productivity. For example, feeding by 18 adult pea aphids over 10 days may completely eliminate primary productivity of young pea plants (Barlow *et al.* 1977) and feeding by 50 adults may reduce the relative growth rate of the plant by 118% over the same time (Barlow & Messmer 1982). By extracting phloem sap and water from plants, aphids also reduce the flow of phytosynthates to the roots, and therefore, to nodules. Formation and maintenance of root nodules may require as much as 17% of photosynthate produced by a pea plant (Minchin & Pate 1973). High aphid densities on both seedling and mature pod-bearing plants also reduce root nodule growth and efficiency of nitrogen fixation by the symbiotic bacterium (Sirur & Barlow 1984). Many of these observed effects were closely related to aphid density, supporting the view that pea aphid feeding damage results from a drain of nutrients, rather than an injection of a toxic salivary secretion (Mittler & Sylvester 1961).

Field pea is grown under very variable conditions in Ethiopia being cultivated in mid-altitude (1800-2200 m a.s.l.), average rainfall of 740 mm and high altitude (> 2200 m a.s.l.), average annual rainfall of 900 mm entirely under rainfed. Field pea is dependent on the rainy

season (June - September) for initial growth and subsequent flowering. Early stoppage of rain during flowering, coupled with poor moisture conditions, render the plants extremely susceptible to attack and damage by aphids. In the highlands (> 2200 m a.s.l.) with sufficient rainfall, aphids have never been a problem. Since 1980, aphid infestations of varying intensity, particularly in the mid-altitude areas have been present each year. Pea aphids feed in aggregations in the upper canopy of field pea plants on the growth tips of leaves, where they cause leaf yellowing, stunting and even plant death.

In western Washington, Getzin & Yencho (1985) estimated total expenditures for pea aphid control ranging from $246,000 to $492,000 per year. Maximum yield losses of up to 35.7% due to pea aphid on pea have been reported in India (Bhatnagar 1996). Outbreaks of pea aphid occur each year in Ethiopia causing considerable yield loss. Incidence of the pest is highest from flowering to maturity (Ali 1999). Aphids extract large amounts of sap from tender stems, flower parts, and unpinned pods, causing reduced growth and yield.

In Ethiopia avoidable yield loss in field pea reach up to 70%, with an average of 36.8% in different regions and under different farming systems (IAR 1987, Beyene & Gebremedhin 1989, Ebsa, Kelbesa & Kiros 1996, Ali 1995 and 1997). From yield loss assessments from four cultivars in Bale region, Ebsa *et al.* (1996) reported a mean yield loss of 28% (Dadimos), 31% (Tullushenen), 50% (G22763-2C) and 53% (local cultivar).

The pea aphid is an important pest of field grown peas in Sweden (Bommarco 1992) and France (Girousse & Bournoville 1994). The aphid is autoecious on various prennial herbaceous legumes. The pest has also been of considerable economic importance in the production of alfalfa forage and seed throughout many areas of the United States. An early

report (1954) estimated losses of alfalfa hay production at 4.1% due to pea aphids. Based on the production acreage at that time, this represents approximately 60 million dollars annually.

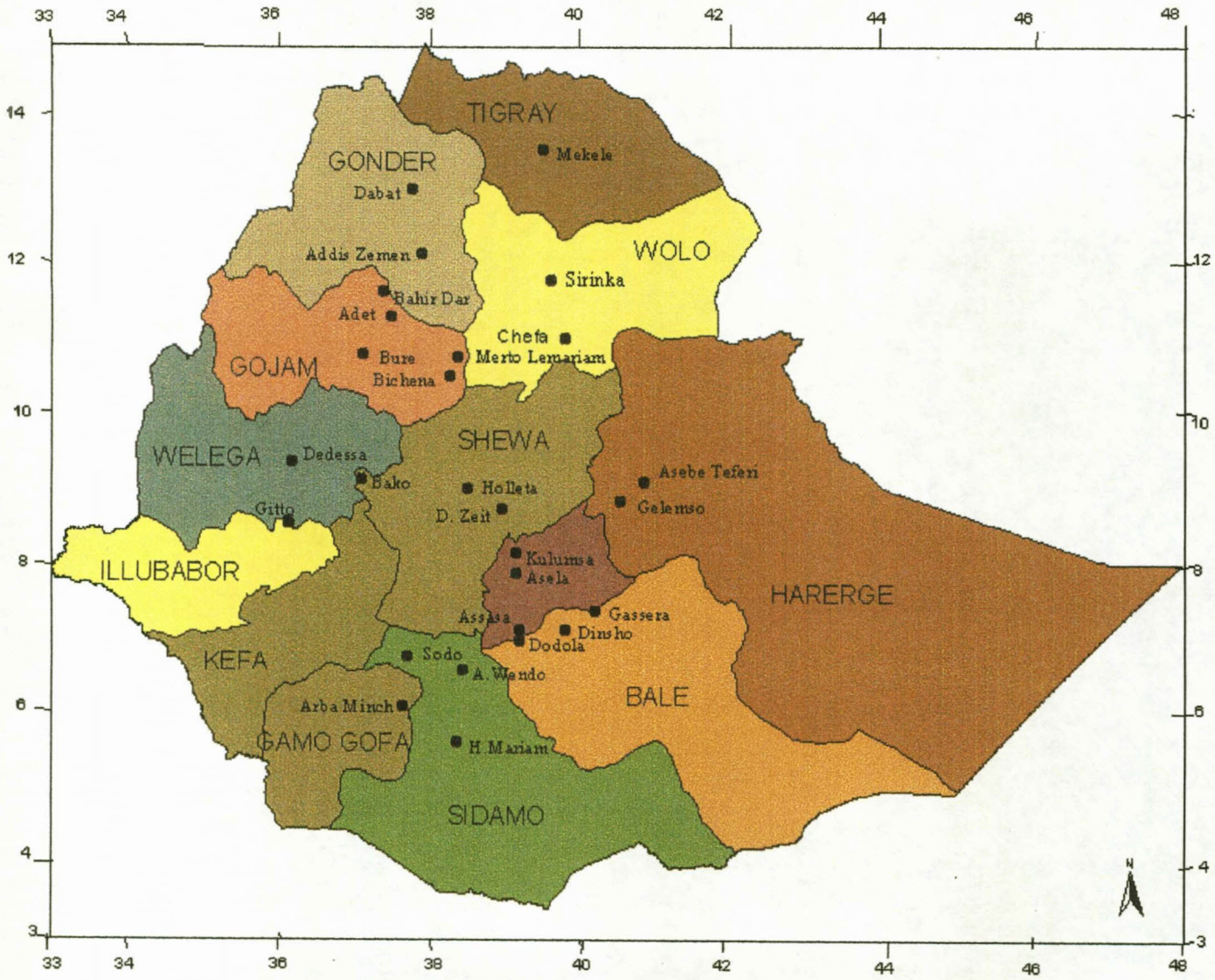
1.3.1.1 History, Distribution and Host Plant

The pea aphid is a cosmopolitan species first noticed in Europe and subsequently recorded from North America (before 1882) (Pickering & Gutierrez 1991), Asia (Bhatnagar 1996, Voronina 1985, Ghani 1971), Middle East (Rassoulian 1992), East Africa (Autrique, Starý & Ntahimpera 1989), Australia (in 1980) (Milne 1986), Argentina and Peru, where it has become a serious pest of alfalfa, and Chile (Starý, Gerdoing, Norambuena & Remaudière 1993). Very recently the pea aphid was recorded on alfalfa in Brazil for the first time (Sousa-Silva, Pachec & Ilharco 1998).

The pea aphid is a widely distributed pest feeding on at least nine genera from the family Leguminosae (Blackman 1974). Preferred hosts are peas, *Pisum sativum* (L.), alfalfa, *Medicago sativa* (L.), clover, *Tripholium partense* (L.), vetch, *Vicia villosa* L. and lentils, *Lens culinaris* Medikus (Gyrisco 1958, Fobers & Frazer 1973, Maiteki, Lamb & Ali-Khan 1986). 'Sweet' lupins in Germany are also attacked by *A. pisum* (Thieme 1997). The pest was also recorded on soybean and haricot bean by Schmutterer (1971) in the western part of Ethiopia. The potential for economic injury by the pea aphid has already been established for peas (Maiteki & Lamb 1985, Yencho, Getzin & Long 1986) and alfalfa (Cuperus, Radcliffe, Barnes & Marten 1982, Wilson & Quisenberry 1986). Pea aphid distribution is generally congruent to that of its host plants, largely Leguminosae.

The present known distribution of the pea aphid in Ethiopia is summarized in Figure 1.1, which has been compiled from surveys undertaken by many authors. Figure 1.1 shows that the aphid is widespread in the country extending from north through the southern and

Figure 1.1 Map showing the distribution of *Acyrthosiphon pisum*



eastern regions', and almost the entire central part. Broadly speaking most of the known localities coincide closely with the distribution of the main pea, lentil and grasspea growing areas of the country. It seems reasonable to conclude that the aphid occurs whenever and wherever these crops are cultivated, with very high populations in the mid-altitudes (1500-2200 m a.s.l.). Ali (1986) notes that pea aphids regularly alight on faba bean, but did not appear to be damaging the plants. This is despite faba bean being successfully used as a host for laboratory studies elsewhere (Mackay & Wellington 1975, Bai & Mackuaer 1990, Sandström 1996, Atanassova, Brookes, Loxale & Powell 1998). It is a common species in southern Ontario living on a number of wild and cultivated legumes, but it is particularly abundant on alfalfa.

Geographic variation in the biotype composition of pea aphid populations has been noted in four distinct studies (Markkula & Roukka 1971, Lamb, Mackay & Gerber 1987, Auclair & Aroga 1987, Sandström 1994). Smith & Mackay (1989) noted that the northerly clones of pea aphid in Canada differ from southerly ones in photoperiod responses. Several biotypes of the pea aphid exist (Sorensen, Wilson & Manglitz 1972). Cartier (1963) observed 23 biotypes, while Hughes & Bryce (1984) reported only two to occur on Australian lucerne. Harrington (1945) and Cartier (1959) described biotypes of pea aphid based on their size differences and differential rates of reproduction on 3 varieties of peas, while Auclair & Aroga (1987) noted differences in tolerance of temperature changes. Winged forms are abundant through late spring and summer in Canada. On alfalfa, Lamb & Mackay (1979) found more than 40% of the larvae bearing wing buds. Dunn & Wright (1955) have observed similar levels of winged-form production in England.

Two of the most important aphid pests of alfalfa, the pea aphid and the blue alfalfa aphid (*Acyrthosiphon kondoi)* were introduced into North America. The pea aphid was introduced from Europe in 1877 and the blue alfalfa aphid from Asia in 1974. Both aphid species occur abundantly as early-season and late-season pests of alfalfa in North America (Harper, Miska, Manglitz, Irwin & Armbrust 1978, Flint 1985, Lamp, Liewehr, Fuentes & Dively 1994). These two species are very similar in size and morphology (Lamp *et al.* 1994, Losey 1996). The pea aphid has also been recognized as a pest of highland legumes of field pea, lentil and grasspea in Ethiopia (Crowe *et al.* 1977, Ali 1986), but it was sporadic problem only briefly referred to in the literature before the last one decade. It is apparently of economic importance in Kenya and Burundi (Eastop 1953).

The faba bean (=broad bean) is considered the common host plant of pea aphid. Birch & Wratten (1984) compared the performance of the pest on 22 wild *Vicia* species and reported it to be the most successful species, in comparison to *Aphis fabae* and *Megoura viciae*, both in terms of higher potential increase and widest host range. Over the years, damage of a more or less serious nature has occurred in mid-altitude areas, where the aphid is generally regarded as one of the major insect problems facing field pea and lentil farmers.

Aphids in general can be divided into two different groups with respect to their life cycle, *i.e.* the non-host alternating (monoecious) species which feed on the same perennial or herbaceous plant species all year round, and the host-alternating (hetereocious) species which migrate between the primary, mostly woody, winter host and one or more species of secondary herbaceous plants during summer.

1.3.1.2 Reproductive biology

Most aphid species reproduce both sexually and asexually, with several generations of parthenogenesis. In temperate regions, sexual forms of most aphid species usually appear in autumn, when the daylength gradually decreases and the temperature drops. Marcovitch (1924) showed that the population of sexual forms in *Aphis forbesi* (Weed) is related to photoperiod. This was the first report of photoperiodism in animals. Daylength has since turned out to be an important factor in the induction of sexual forms of many aphid species, *e.g. A. pisum* (Kenten 1955, Lamb & Pointing 1972).

The pea aphid in northwest and central Europe is predominantly holocyclic, possessing a number of strains that are each differently adapted to living on a range of leguminous species (Muller 1962). The females that hatch from the eggs are the first of a series of summer parthenogenic generations. In the fall, photoperiod and temperature stimulate the parthenogenic females to produce a single sexual generation (Lamb & Pointing 1972).

Dixon (1987) provides a more comprehensive account of reproduction in aphids. Parthenogenic reproduction evolved in aphids in the Permian age (200 million years ago) and has been of paramount importance in determining their population structure and high rates of increase. As early as 1745, Bonnet (as cited by Dixon 1985) proved beyond doubt that aphids may propagate without fertilization and continue to do so for as many as 10 generations. Then, under certain conditions, winged or wingless males appear and copulate with wingless oviparous females, giving rise to cyclical parthenogenesis that is characteristic of most aphids. Huxley (1858) was the first to show that if aphids were kept warm and supplied with food they could reproduce parthenogenically without deterioration, apparently

indefinitely, which is supported by the existence of many anholocyclic species of aphids. It was not until 1924 that Macrovitch demonstrated the role of photoperiod in the induction of sexual forms. It was in 1924 that Macrovitch demonstrated the role of photoperiod in the induction of sexual forms. In sexual reproduction the telescoping of generations is absent, since an aphid that must mate cannot begin to mature its embryos before they are born. On the other hand, the embryos of partenogenically-reproducing aphids can have embryos developing within them. A few species are oviparous, with the eggs hatching immediately after they are laid (Hill Ris Lambers, 1950 as cited by Dixon 1987).

The pea aphid uses a variety of herbaceous legumes as host plant, and is not obliged to alternate between a winter and a summer host (Bommarco & Ekbom 1996). In Northern Hemisphere areas such as Canada, England, Finland and Sweden it overwinters exclusively as diapausing eggs on perennial legumes (Bronson 1935, Dunn & Wright 1955, Markkula 1963). In the spring fundatrices hatch from the eggs and give rise to a parthenogenically reproducing population, some of which are winged and will eventually migrate to annual legumes such as peas. In the pea field a parthenogenically reproducing population develops rapidly. Infestations during flowering and early pod stages can lead to plant deformation and high yield loss (Barlow *et. al* 1977).

Parthenogenesis in aphids appears to allow for no genetic recombination (Blackman 1978), but permits the rapid reproduction of well-adapted genotypes. All parthenogenically produced offsprings are genetically identical to their mother, barring mutations (Blackman 1981). The sexual generation provides the opportunity for recombination and the synthesis of genotypes. It is believed that the pea aphid is anhocyclic in Australia, where sexual

morphs have not been observed in nature (Hughes & Bryce 1984). Mackay, Lamb & Hughes (1989) concluded that sexual reproduction is not common among Australian pea aphids.

1.3.1.3 Biology

Kennedy & Stroyan (1959) reviewed the general biology of aphids. Carteir (1960), Sylvester & Richardson (1966), Murdie (1969a 1969b), Frazer (1972), Siddiqui, Barlow & Randolf (1973), Mackay & Wellington (1975), Campbell & Makuaer (1975), Dixon (1985), Sandström (1994), Chakraborty & Dutta (1998), Damte (1999) and many others provided a detailed account of many aspects of *A. pisum* biology. In the USA, fall populations of *A. pisum* virginoparous adults begin producing sexual offspring when the photoperiod approaches ca. 10:5D: 13:5L. Mated females (ovipare) oviposit close to the crowns of alfalfa plants (Bronson 1935). Stem mothers (fundatrices), which develop from overwintered eggs and all subsequent generations throughout the growing season, are parthenogenic and viviparous. Parthenogenic reproduction, combined with a rapid rate of development, allows the aphid population to reach levels causing economic injury in a short time. Harper *et al.* (1978) provides an exhaustive bibliography of *A. pisum* bionomics.

The pre-reproductive period of pea aphid reared on broad beans in greenhouse at $20^0 \pm 0.05^0$C and 70-80% RH varied from 7.6 days (Frazer 1972) to 8.9 days (Cartier 1960) in Canada. Sandström (1994) reported a similar pre-reproductive period on pea cultivars. A parthenogenic female produces between 83.7 nymphs (Siddiqui *et al* 1973) on field pea in greenhouse at 20^0C and 60 ± 10% RH to 101 on alfalfa at 14.8^0C, 50-70% RH (Campell & Mackauer 1977), although individuals may produce as many as 128 on pea at 20^0C (Markkula & Roukka 1971). Average total fundatrix (stem mother) fecundity on alfalfa at 16^0C and 50-70% RH, was 52 nymphs (Bommarco & Ekbom 1995).

Maximum mean daily fecundity of pea aphid on peas ranged between 8 nymphs (Siddiqui *et al.* 1973) and 12 nymphs per day at 20^0C and 60-70± 10% RH (Murdie 1969b), while it was only 5.3 to 6.8 nymphs under the same environment (Sandström 1994). In India, Chakrabotry & Dutta (1998) reported the mean duration of 1.2 days for first and second instars, 2.0 and 2.1 for third and fourth instars, respectively on peas. The adult had pre-reproductive, reproductive and post-reproductive periods of 3.0, 12.6, and 2.4 days respectively. The life cycle was completed in 9.4 days with total life span and adult longevity of 24.4 and 18.0 days respectively. On alfalfa, Campbell & Makauer (1975) recorded an average nymphal period of 7.5 days (apterae) and 8.2 days (alatae), with mean duration of four nymphal instars as 1.89, 1.69, 1.74, and 2.23 days at 20^0C. The average nymphal periods were much lower at a temperature of 26^0C, with an average of 5.4 days for apterae to reach an adult stage. At 10^0C it took 23 days for apterae and 26.5 days for alatae. The developmental duration of the pest varies considerably under different conditions of temperature, humidity, biotypes and host plant. At constant temperatures under controlled conditions the nymphal stage requires about 6 days at 27.5^0C and 18 days at 10^0C for apterae, while it took 7 and 20 days respectively for alate forms under similar conditions (Hutchison & Hogg 1984). Other workers (Campbell & Mackauer 1975, Frazer & Gilbert 1976) have reported developmental times within this range. However, the magnitudes of these trends differ. For example, Campbell & Mackauer (1975) studied populations of *A. pisum* from Kamloops in Canada, which has a warm dry climate. They found that the time needed to reach adult stage at 10^0C was 23.0 days for apterae and 26.5 days for alates.

The total life span of pea aphid may range from about three weeks to longer, depending largely on environmental conditions. According to Mackay & Wellington (1975),

the life span on faba bean at $20^0 \pm 1^0$C was 29 and 31 days for apterae and alatae respectively. Frazer (1972), however, reported an average of 25 days with the highest of 45 on faba bean ($20^0 \pm 0.5^0$C, 70-80% RH). These differences may have been partly due to clonal differences arising from different biotypes, but there would also have been slight differences in rearing conditions or the host plant used. Different varieties of host plants and biotypes of aphid species have been shown to produce different fecundity and survival data. Therefore, the biology of pea aphids is not easily compared because of combined effects of different plant varieties and aphid biotype.

Temperature dependent growth rates have been calculated for each of the immature stages of *A.pisum*. Laboratory studies provide estimates of both lower and upper threshold for development. Hutchison & Hogg (1984) reported 2.8^0C and 26.0^0C lower and upper threshold, respectively for development on alfalfa. Developmental thresholds of North American clones of *A. pisum* range between 2.5^0C to 5.6^0C (Campbell, Frazer, Gilberet, Gutierrez & Mackauer 1974). The optimum temperature for rapid development ranged from 23.3^0C to 27.8^0C (Lamb *et al.* 1987), 19-20^0C (Kenten 1955) and 11.9-19.6^0C (Morgan, Walters & Aegerter 2001). Harrison & Barlow (1972) and Siddiqui *et al.* (1973) noted that temperatures between 25 and 30^0C adversely affected development and survival of the pest. The above comparison reflects an important adaptation of *A. pisum* to cool climate. Low threshold allows the pest to exploit its host earlier in a cool climate. The subsequent faster rates of development at low temperature aid in minimizing time necessary to reach adult stage or time taken to first reproduction.

1.3.1.4 Mortality

A number of biotic and abiotic factors affect pea aphid survival, which determine what fraction of the potential rate of increase is realized. They respond to cues that signal seasonal trends in food quality and weather, by developing particular morphs that are well adapted to survive the seasonal change in habitat quality (Dixon 1985). However, unpredictable short-term changes in weather and food quality can result in mortality (Cartier 1972, Watt & Dixon 1981). Similarly natural enemies can also have dramatic effect on survival (Cavalloro 1983), especially that of young aphid instars (Campbell & Mackauer 1975, Sequeira & Mackauer 1988).

The sole cause of death in reproducing aphids in a study by Frazer (1972) was associated with the birth of nymphs. Some females are unable to deposit a dead nymph or the succeeding one and consequently the aphid becomes engorged with developing embryos and dies within 3 - 4 days. Frazer (1972) also noted a high incidence of nymphal mortality when the nymphs were unable to free themselves from the embryonic membranes, when reared at 30^0C, but no effect on the mothers were reported. Mackay & Wellington (1975) reported that mortality was highest during the first and the final molts. Pre-adult mortality of the apterae ranged from 5 - 10%. The same authors reported mortality among the alatae to be higher than among the apterae just prior to the final molt. According to Pickering & Gutierrez (1991) the mortality of pea aphid by entomopathogenic fungus (*Pandora neoaphidis*) was estimated to be 25% and 5.2% by parasitoids in California, USA. During outbreaks, usually occurring in April-May or September-October, *Pandora* controlled *A.pisum* below economic levels only during wet periods when humidity was sufficiently high for its transmission. According to Lamb *et al.* (1987) less than 8% of the pea aphids died at

temperatures of 25^{0}C, before reaching adulthood. However, mortality was 21% at 27.5^{0}C under laboratory conditions.

1.3.1.5 Behavior

The physiological mechanisms controlling the production of pea aphid alatae have been well studied. Sutherland (1969a) has shown that physical contact among adult pea aphids initiates alate production. Deterioration of the host plant has a similar effect (Sutherland 1969b, Dixon 1985). Pea aphid is capable of dispersing in significant numbers over long distances (Berry & Taylor 1968, Taylor 1979) and Smith & Mackay (1989) estimated migratory distance of more than 300 km from more southerly latitudes into southern Manitoba, Canada. Aphid flight speeds range from 0.8 to 3.3 km/h (Robert 1987).

The behavior of pea aphid is well known. Flight is one of the most important aphid behavior patterns. Dixon (1985) presents a detailed account. He notes that adults are capable of making short as well as long flights and that there are distinct morphs that differ in both their flight behavior and body structure. These latter features are more pronounced in host alternating species. In many aphids, including pea aphid, the parthenogenetic females show alary dimorphism, i.e. they can be either winged (alate) or wingless (apterous). Both green and red forms are found (Sandström 1996, Losey, Ives, Harmony, Ballantyne & Brown. 1997) and these forms are apparently of genetic origin, since they are stable in clonal lineage (Muller 1971). Maiteki & Lamb (1985), Yencho *et al.* (1986) and Bommarco (1992) reported that, in spring, migrants of pea aphid fly to annual legumes such as peas where it is an important pest in the northern areas of Sweden. Bommarco & Ekbom (1996) also showed that fundatrices of the pea aphid produce 46% and 28% of the green and red forms of winged offspring respectively. The red forms dominate on clover (67%), whereas the green forms

dominate on alfalfa (80%) (Sandström 1994, 1996). In Germany, Daebler & Hinz (1987) reported that the green form was predominant, with the red form accounting for only 7.4%. Twelve individuals at one site represented the yellow aphid population. On peas all aphids are green.

The pea aphid is also capable of defense against natural enemies. The aphid will kick or attack when approached, attempt to walk away or drop off the plant, depending on the relative size of the attacking parasite or predator (Dixon 1958, Chau & Mackauer 1997, Losey 1996, Losey & Denno 1998b). This kind of escape is often accompanied by the release of an alarm phermone that alerts nearby aphids. The pea aphid shows a heightened response to crowding when developing on mature leaves and can even produce alatae solely in response to changes in host quality (Smith & MacKay 1989).

1.3.2 Ascochyta blight

1.3.2.1 Importance and distribution

Ascochyta blight (Nene, Hanounik, Qureshi & Sen 1988) causes the most serious and widespread fungal foliar diseases of cool season food legumes (faba bean, field pea, chickpea, lentil, and grasspea). Ascohyta blight of chickpea, faba bean and lentil are caused by *Ascochyta rabiei*, *A. fabae* and *A. fabae f. sp. lentis*, respectively. All species of *Ascochyta* are seedborne and survive for a year or more on infested crop residue. Three fungi can cause the ascochyta disease complex of field pea: *Ascochyta pisi*, *Mycosphaerella pinodes* (the perfect stage of *Ascochyta pinodes*) and *Phoma medicaginis* var. *pinodella.* These diseases are widespread throughout the world, particularly in the temperate areas of Europe, North America and New Zealand (Hagedorn 1985). There are numerous reports that *M. pinodes* is the most destructive of these species (Wallen 1974, Hagedorn 1984, Zimmer &

Sabourin 1986, Gorfu & Beshir 1994, Gorfu 2000). The disease is the most serious on field pea grown in Ethiopia and elsewhere, especially in temperate and sub-tropical areas of the world (Pumithalingam & Holiday 1972, Lawyer 1984, Allard, Bill & Touraud 1993). This is particularly true in France (Tivoli, Beasse, Lemarchand & Masson 1996), UK (Girsch 1988, Nasir & Hope 1991), Australia (Bretag, Keane & Price 1995), Canada (Warkentin, Rashid & Xue 1996) USA (Kraft, Dunne, Goulden & Armstrong 1998) and Ethiopia (Gorfu & Beshir 1994).

The disease causes spot-like necrosis on aerial organs and causes yield and seed quality losses (Allard *et al.* 1993, Garry, Jeuffory & Tivoli 1998). The disease infects pea seedlings as they emerge causing girdling stem lesions that reduce field population and increase lodging (Ryan, Staunton & Cassidy 1984). Later it also causes necrotic lesions on leaflets and stipules and, in exceptional circumstances, abscission of the leaflets (Hagedorn 1984, Tivoli & Lemarchard 1992). The disease is responsible for yield losses of up to 30% when humidity is high (Allard *et al.* 1993). Ascochyta blight typically appears in a rapid and explosive way. In France it is epidemic every year (Tivoli & Lemarchand 1992). The disease infects all aerial portions of the pea plant (leaves, stems and pods), resulting in numerous lesions and extended necrosis. Tivoli *et al.* (1996) explained yield losses due to this disease on pea by reduction in the number of seeds per stem and seed size. They also reported that the harvest index and total biomass were lower in diseased plants and seed yield was reduced by 40% in diseased plots.

Reports of crop losses in peas by ascochyta blight (Ali, Nitschke, Dube, Krause & Cameron. 1978, Lawyer 1984) indicate yield reduction of up to 70% when infection is severe. Ascochyta blight, caused by *M. pinodes*, infects most pea crops in Australia,

contributing to a yield penalty of 10 - 20%, and which in some years can result in total crop loss on individual farms (Bretag *et al.* 1995). In France yield losses can reach up to 40% in diseased plots (Tivoli *et al.* 1996). In the USA, Sell & Aakre (1993) estimated that, under moderate to severe infections, ascochyta blight could reduce yield by 20 - 50%. Gorfu (2000) reported similar results in Ethiopia.

M. pinodes attacks the maturing pods from which the fungus invades the developing seeds. Heavily infected seeds are 'stained' by the fungal lesions and some of the unstained seeds may harbor infection. The pathogen overwinters mainly in soil and on crop debris (Sheridan 1973), and can develop rapidly during periods of wet weather and moderate temperature. Typically the first sign of disease is a large number of small blue-black spots on aerial organs. They enlarge quickly, coalescing to form necrotic lesions under favorable conditions, and leading to premature host senescence (Kerling 1949). In the field, infection and fruit body formation starts at the plant base and progresses upward (Roger & Tivoli 1996). Healthy leaves may be infected by pycnidiospores splashed by rain, or by ascospores dispersed by wind.

Garry, Tivoli, Jeuffroy & Cithavel (1996) demonstrated that ascochyta blight alters carbohydrate metabolism, protein remobilization and free amino acid translocation from hulls and leaves. These workers also noted that the disease reduced carbohydrate and nitrogen content in seeds, and in case of high disease severity the carbohydrate nitrogen ratio in the seed was also affected.

1.3.2.2 Epidemiology

The fungus can occur in all parts of the seed. The amount of infected plant debris, frequency of seed transmission, which varies over genotypes according to their resistance,

and wet conditions are factors that determine the onset of ascochyta blight (Beauchamp, Morrall, & Slinkard 1999). The frequency of transmission from infected seed to seedling is low, especially at moderate to high soil temperatures. Frequent rainfall can cause a major epidemic of ascochyta blight in a pea field if inoculum is present. The source of primary inoculum may be either infected seed or stubble. Conidia spread from infected stubble to plants and from plant to plant within a crop. Subsequent disease development occurs by transmission of the pathogen from seed to the epicotyl and random dispersal of conidia from infected stubble (Clulow, Lewis & Matthews 1991). The cold and wet conditions during crop establishment in the winter are possibly favorable to pathogen establishment on the slowly developing seedlings.

Long-distance spread of ascochyta blight is through the sowing of infected seed in previously disease-free areas. Infected seed provides the fungus with an important survival mechanism. Kaiser (1989) reported that the storage of infected lentil seeds for 4 years at 20°C, 5 - 18 °C, 160 °C and 196^{0}C did not adversely affect the pathogenicity of the fungus. It survived for more than 3 years in infected pods and seeds at 4 - 5^{0}C or in a shelter outdoors, and for 1.5 years on the soil surface, but lost its viability within 29 weeks at a soil depth of 16 cm.

Clulow *et al.* (1991), Clulow, Lewis & Matthews (1992) and Nasir, Hope & Ebrahim-Nesbat (1992) have described the infection process by pycnidiospores (germination, appressorial formation, and penetration). Temperature and leaf wetness are major factors affecting disease and fruiting body development for many aerial pathogens (Huber & Gillespie 1992). Ascochyta blight of pea was reported between 10 °C and 20^{0}C, but the optimum temperature for infection was 15 - 18^{0}C (Wallen 1965) or 20^{0}C (Bretag 1991). The

first symptoms may appear 48 - 72 h after inoculation (Allard *et al.* 1993), but at sub-optimum temperatures, a larger period of leaf wetness was required for infection (Bretag 1991 as cited by Roger, Tivoli & Huber 1999).

Inoculum concentration, temperature and moisture duration were shown to be important environmental factors affecting infection, disease development and production of secondary inoculum (Roger *et al.* 1999). The life cycle of the pathogen is rapid under optimum moisture conditions. The optimum temperature for disease development was 20^0C, but wide range temperatures (15°C, 20°C and 25^0C) allowed rapid development of the disease. Disease progress and pycnidia developments are reduced with inoculum availability.

For polycyclic airborne fungi, the ability to produce secondary inoculum affects subsequent infections and progress of the disease. *M. pinodes* produced new pycnidia in 3-4 days at optimum temperatures (15 - 20^0C). The latent period for *Ascochyta rabiei* on chickpea (5.5 days) (Trapero-Casa & Kaiser 1992), *A. fabae f. sp. lentis* on lentil (6 - 7 days) (Pederson & Morrall 1994) and *A. fabae* on faba bean (8 - 10 days) (Wallen & Galway 1977) are comparatively longer. For spring pea crops, the period of leaf wetness can be infrequent and temperatures higher, preventing the disease and pycnidia from developing rapidly on the leaves. Spread of infection from leaf to leaf by pycnidiospores in rain-splash droplets is easier in the winter crop than in the spring crop. Under particularly unfavorable climatic conditions, there may be an irreversible lag in epidemic development, regardless of conditions for pycnidiospore dispersal and infection (Royale 1994, Lovell, Parker, Hunter, Royle & Coker 1997). However, *M. pinodes* could develop on all parts of the pea plant and spread by wind-dispersed ascospores produced on senescent pea leaves (Roger & Tivoli

1996). Field samples of pea tissue infected by *M. pinodes* usually bear numerous pycnidia of the asexual stage (*A. pinodes*) on green and senescent parts of the growing plant, and a great number of perithecia, formed as a result of fertilization of sexual hyphae, on the lower senescent leaves of the plant (Wroth & Khan 1999).

Experimental results obtained under controlled conditions contribute to the understanding of the development of *M. pinodes* epidemics on pea crops. Field epidemics depend on disease development and the length of the incubation and latent periods which control inoculum availability. The disease can develop within a few days under favorable moisture conditions and over a wide range of temperatures, explaining the explosive eruption of the disease in rainy conditions when inoculum is available. Currently available fungicides must be applied before the pathogen invades host tissue to ensure successful control. Use of thresholds for low, moderate and severe levels of disease can allow quantitative characterization of the epidemic, and may contribute to disease control strategies. Prediction of dry and wet periods in the field allows adjustment of fungicide application (Gallois, Le Breton & Martin 1983). The host growth stage (Pederson & Morrall 1994) and environmental and nutritional conditions (Roger & Tivoli 1996) may also affect disease and fruiting body development.

1.4 Control

1.4.1 Host Plant Resistance

Current methods for controlling pea aphid mostly rely on insecticides, high cost inputs, and environmental hazards. The use of insect resistant cultivars is the most economical, easily applicable and environmentally sound alternative to insecticides and a key component in an integrated pest management system.

Host-plant resistance has significant advantages over other pest control strategies in situations where: i) an insect is exposed for only a brief period of its life cycle; ii) The crop is of low economic value; iii) the pest is continuously present and is the single most limiting factor in successful cultivation of the crop in a wide area; iv) other controls are not available. These four conditions all apply to pea aphid on field peas in Ethiopia.

Painter (1951) classified plant resistance to insect pests into three inter-related components: a) 'non-preference' for oviposition, food or shelter (often now re-named as 'antixenosis'), b) 'antibiosis', where the plant adversely affects the biology of the insect, and c) 'tolerance', where the plant has the ability to withstand infestation, often through repair or recovery. A fourth type of resistance, 'pest avoidance', which is a tendency to escape infestation, should also be considered. This latter resistance component was added to Painter's classification by Russell (1978).

Painter (1951) also identified various practical aims of screening for resistance: a) to use plant resistance as the principal control method for a pest, b) to develop resistance as an aid to other measures, and c) to safeguard against the release of particularly susceptible varieties. Russell (1978) stated that it is not always necessary or desirable to breed for a very high level of resistance. Incomplete resistance has often given an adequate level of control in the field, particularly when such resistance has been supported by other control measures.

Plant resistance to insects has proved especially valuable in most parts of the world where individual land holdings are too small to permit the economical use of insecticides and where growers are not familiar with their use. More than a hundred years ago Sindley (1831) was the first to report on an apple variety, Winter Majition, which is resistant to the wooly aphid, *Eriosoma lanigerum* (Hausm.). The first extensive observations of winter wheat

resistance to hessian fly were reported by Wickson (1880), Woodworth (1891) and Kellner (1892) who were working in California. Much of the impetus to investigate sources of resistance in plants to insect attack come from Painter and his colleagues in Kansas, who were particularly concerned with selecting resistant genotypes of crop plants and with breeding programs for the development of resistant commercial varieties.

Earlier, Russell (1978) reported many successful examples of resistant varieties. For example, varieties with resistance to hessian fly, *Mayetiola destructor* (Say), have increased the value of wheat crop in North America by several millions of dollars annually for more than sixty years. Stem sawfly, *Cephus cinctus* Nort, is no longer a serious pest of wheat in those parts of Canada and the USA where resistant varieties have been grown for several years.

Many workers reported field pea varietal resistance to pea aphid. The first observation on the resistance of pea plants to pea aphid was published as early as the 1920's (Russell & Morrison 1924) and Blanchard & Dudley (1934) first observed resistance in alfalfa to the pea aphid. Since then, many instances of cultivar resistance to the pea aphid have been reported, ranging from studies which simply note the greater numbers of *A. pisum* on one cultivar (Searls 1932, 1935, Markkula & Roukka 1971) to others which investigate the basis of the differences in aphid populations (Bintcliff & Wratten 1980, Bieri, Baumgartener, Bianchi, Delucchi & von Arx 1983, Downes 1994, Morgan *et al.* 2001). In North America, many alfalfa cultivars resistant to pea aphid were developed during the 1970's (Sorensen, Byers & Horber 1988). The mechanisms of resistance to aphid have also been described as antibiosis (Dahms & Painter 1940, Laughlin 1965, Markkula & Roukka 1971, Campbell & Mackauer 1977, Leather & Dixon 1984, Holt & Wratten 1986, Mackay &

Lamb 1988, Soroka & Mackay 1991, Holtkamp & Clift 1993), antixenosis (Ellsbury, Pant & Knight 1985, Holt & Wratten 1986, Haltkamp & Clift 1993), and tolerance (Newman & Pimentel 1974).

According to Searls (1932, 1935) the pea aphid prefers field pea varieties with deep-green foliage, although the studies of Cartier (1963) demonstrated that migrants and colonies were most numerous on a variety with yellowish-green foliage and sparsest on a variety with deep-green foliage. Harrington (1941) and Maltais & Auclair (1957) reported differences in terms of pea aphid damage among pea varieties. After testing 103 varieties of peas against 3 biotypes of pea aphid, not a single line proved resistant to biotype 1 a. However, all varieties were fairly or highly resistant to the biotypes 1 b and 16 (Markkula & Roukka 1971). They reported that biotype 1b produced only a few progeny on cvs. Onward, Reform and Perfection while biotype 16 did not reproduce on these varieties at all. Kehr, Manglitz & Ogden (1968) and Painter (1968) reported resistance of alfalfa to pea aphid. In Australia, Salisbury, Downes & Muller (1985) reported that the two Lucerne varieties, CUF101 and WL514 (originating from America) show high level of pea aphid resistance.

Aphids are the most frequent insect group for which formation of host-adapted races (biotypes) and rapid adaptations to resistant crops have been observed (Caillaud, Dedryver, Di Pietro, Simon, Fima, & Chaubet 1995). For instance, the specialization of the pea aphid on herbaceous Fabaceae has been reported in Europe by Muller (1980) for populations living on *Vicia* species, peas, alfalfa and red clover, and in North America by Via (1989, 1991) for populations found on alfalfa and red clover. When considering resistance of plant varieties to aphids, the distinction of new biotypes is based on their ability to feed and damage plants resistant to some other biotypes (Claridge & Den Hollander 1983). These variations in

aphid/host-plant relationships are important as they may alter breeding programs for crop resistance to aphids.

In the pea aphid, the variability in host-plant interactions may occur at the local level, either between different plant species (Via 1991) or between clones on a single host (Sandström 1994). Bournoville (1981) showed a similar magnitude of variation in reproductive rate among clones originating from the same field of alfalfa and among clones collected from geographically distant alfalfa fields when tested on a susceptible cultivar of alfalfa.

Sandström (1994) tested the susceptibility of 37 cultivars of *Pisum* to different clones of *A. pisum*, and found that pea aphid clones showed little difference among cultivars. The pea aphid clones collected from red clover generally performed relatively poorly on pea cultivars, in contrast to the pea aphid clones collected on alfalfa. According to Soroka & Mackay (1991), resistance of field pea cultivars 'Tipu' and 'Century' in laboratory studies was due to antibiosis expressed as decreased aphid fecundity and longevity of the six cultivars tested. Similarly for *A. pisum* on clover, Zeng, Pederson, Ellsbur & Davis (1993) reported that nymphal production per adult was greater on the 'Tensas' cultivar, than on the N-2 genotype, suspecting that this germplasm may contain a toxin, or that a toxin may be induced in the plants due to aphid feeding.

In several studies, resistance of peas to pea aphid has been investigated, with variable results. Markkula & Roukka (1971) and Soroka & Mackay (1991) found that differences between cultivars were small, whereas Cartier (1963) and Newman & Pimental (1974) reported substantial differences in resistance between certain cultivars. These inconsistencies could be due to the fact that the different screening methods used do not measure the same

components of resistance. Some methods measure antixenosis that affects aphid behavior, while others measure antibiosis that affects aphid physiology. The resistance reactions could also have varied depending on which pea aphid biotype or clone was used in the screening tests (Markkula & Roukka 1971, Frazer 1972). The mechanism of resistance in many crop plants towards aphids is frequently ascribed to the presence of secondary metabolic products in the plant that have toxic or feeding-deterrent effects (Dreyer & Jones 1981).

Among the features that might deter the development of pea aphid on resistant alfalfa cultivars, Dreyer, Jones, Jurd & Campbell (1987) stated that the rate of enzymatic catalyzed depolymerization of the pectin isolated from different alfalfa lines correlates with plant resistance to aphids. The results of Rahbé, Febvay, Delobel & Bournoville (1988) argue that the role of certain secondary metabolic products in host-plant resistance to aphids cannot just be dismissed out of hand. Compounds with a small alkyl residue in the 3-acyl group showed the greatest feeding detergency towards the pea aphid. In most of the early papers on the nutritional origin of resistance to aphids, quantitative differences in nitrogen availability have been stressed as the main resistance factor. Particularly with *A. pisum*, Maltais & Auclair (1957) (as quoted by Rahbé, Febvay, Delobel & Bournoville 1988) have pointed out the positive correlation between the sugar/amino acid ratio in plant tissue and the plant's resistance to the aphid.

According to Auclair (1963) asparagine, glutamine and homoserine are the factors determining the resistance of pea varieties. Banks (1965) states that the fecundity of *Aphis fabae* seems to depend primarily on the quality of the nutrients it gets from its host. More recent work on resistance to pea aphid on two lucerne genotypes by Girousse & Bournoville

(1994), suggests that aspects of phloem physiology other than sap composition, are responsible for lucerne (cv. Lohontan) resistance to pea aphid.

The simple model of van Emden & Wearing (1965), which suggests that partial plant resistance should increase the impact of biological control, has since been further elaborated and experimental results confirming the model have been reviewed (van Emden 1990). According to Gowling & van Emden (1994), disturbance of aphids by parasitoids are probably among the mechanisms, which could be involved in the positive synergism between partial plant resistance and biological control.

For decades geneticists have bred crops with improved characteristics, among them resistance to insect pests. At the same time chemists and entomologists have developed a stream of improved chemical and biological insecticides. Recently molecular biologists have combined the two in a third approach to insect pest control referred to as genetic engineering of crops and which produces insecticidal or antifeedant proteins continuously in the field.

In recent years, a number of different classes of proteins have also been reported to promote toxic effects when ingested by plant sucking Homoptera (Gatehouse, Hilder, Powell, Boulter & Gatehouse 1992, Rahbe & Febvay 1992, Habibi, Backus & Czapla 1993, Powell, Gatehouse, Hilder & Gatehouse 1993). Use of transgenic strategies to produce homopteran resistant crops was therefore conceivable, provided that these proteins could be expressed at the required levels in the appropriate plant tissues, this being primarily determined by the target insect(s) in question.

Recent advances in plant biotechnology, such as the use of trangenic cultivars and marker-assisted breeding to better exploit host plant resistance, have provided new approaches for the management of insect pests. One of the most promising of these is the

development of cultivars that express one of the δ-endotoxin genes from *Bacillus thruingiensis* subsp. *kurstaki* (Roush 1997). A review article lists 18 species of economic importance that have been successfully transformed with Bt gene (Mazier, Pannetier, Tourneur, Jouanin & Giband 1997). This number is continuously increasing, and several transgenic cotton, maize, and potato cultivars have been commercialized since 1995 (Jouanin, Bonadé-Bottino, Girard, Morrot & Giband 1998). Bt cultivars were planted on ≅ 1.2 million hectares in the United States in 1996, and by 1997 plantings had increased to an estimated 1.05 million hectares of Bt corn, 1.0 million hectares of Bt cotton, and 10, 000 hectares of Bt potato, as well as plantings in Australia and Canada (All, Boerma, Parrott, Stewart, Raymer, Rector, Ramachandram, Walker & Treacy 1999). Transgenic cultivars that produce other insecticidal proteins are under development (Carozzi & Koziel 1997). Although transgenic insecticidal cultivars (TIC) are a new innovation, they also represent an extension of one form of classical host-plant resistance that is often called antibiosis.

The coloning of plant resistance genes is an important goal that may soon be achieved. Workers on *Arabidopsis thaliana* (which provides a useful model system) are concentrating on plants with deletion mutations in resistance genes. Other groups, working on tomato, are mapping resistance genes using restriction fragment length polymorphism (North 1990). Projects are now under way in the USA, UK and Japan to map the entire genome of various crop plants, including rice, wheat and barley (Ferrell 1991).

Genetic engineers can address plant/pest interactions at the molecular level. This understanding will enable scientists to devise measures more specific and less harmful to non-target organisms. These measures for pest management could include new synthetic chemicals, as well as transformed plants marketed as proprietary seed and improved

biological pesticides. The important question is how sustainable is this system? Can its sustainability be improved through the adaptation of biologically enhanced IPM programs?

Pests (diseases and insects) can adapt to any management tactic depending on the selection pressure exerted on them, so deployment strategies must be designed and implemented to delay or prevent the breakdown of resistance. The host plant resistant deployment strategies must be integrated into an overall integrated pest management (IPM) program that incorporates multiple tactics (cultural, biological, chemical etc.) to diversify pest mortality sources and reduce subsequent selection pressure on the pests. Pest resistance management must be viewed within the context of IPM. If IPM is successfully adopted and implemented, the objective of resistance management will be automatically achieved. It is worth emphasizing that resistance management is likely to be key to successful IPM in the future and the concept must embrace both the battle against resistance to pesticides (of all kinds), and enhancement of resistance of crop plants to pests.

Although good progress has been made in developing pea cultivars resistant to powdery mildew, such progress is not apparent for other foliar diseases. *Fusarium* wilt resistant cultivars of pea are available and thus host plant resistance offers the best prospect for control of this disease (Hagedorn 1985). Resistance trials to the three *Ascochyta* species (*Ascochyta pisi*, *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella)* have been performed under greenhouse conditions, but no single source of resistance to all three species was found (Ali *et al.* 1978). Previous studies have shown that the population of *M. pinodes* is highly variable and that it can be divided into pathotypes that differ markedly in their virulence on leaves of different *Pisum* species or pea lines and cultivars (Nasir & Hoppe 1991).

Currently, no genetic resistance to *M. pinodes* has been developed in commercial pea cultivars (Kraft *et al.* 1998). No single gene for a high level of resistance to this disease has been found, despite extensive searches within the available gene pool. For example, among 2936 accessions of *P. sativum* screened under field conditions, only five accessions were found to be resistant to *M. pinodes* attack (Kraft *et al.* 1998). The possibility of physiological specialization within *M. pinodes* makes it even more difficult to develop resistant cultivars. Clulow, Matthews & Lewis (1991) reported that numerous pathotypes of *M. pinodes* exist and Nasir & Hope (1991) divided 50 single conidium-derived isolates into 6 pathotypes using 15 pea cultivars. Consequently, it may be necessary to incorporate several resistance genes into a cultivar in order to achieve field resistance to *M. pinodes*. However, several workers (*e.g.* Ali *et al.* 1978, Bretag 1989, Nasir & Hoppe 1997, Baranger & Tivoli 1998, Wroth 1998, Wroth & Khan 1999) have reported the presence of blight resistance in pea against *M. pinodes*. Clulow (1989) reported that several wild accessions of *P. sativum* were resistant in a controlled environment.

1.4.2 Biological control

1.4.2.1 Pea aphid

The use of biological control agents to prevent or minimize crop losses from insect pests is not a new concept. There are three general approaches to biological control, namely importation, augmentation and conservation of natural enemies. Each of these techniques can be used either alone or in combination in a biological control program. Biological control occurs naturally to suppress potential crop pests, and under optimal conditions, can be effective in bringing about their economic control. In many instances, predators, parasitoids and other biological antagonists break down, necessitating artificial means of control through

the use of materials such as synthetic chemical pesticides. The increasing wide spread use of pesticides, many of them having a broad spectrum of activities, have undoubtedly led to a decrease in the effectiveness of natural pest control mechanisms. Attempts to break this chemical pesticide cycle have focussed on concepts such as IPM programs, and more recently on what is referred to as sustainable agriculture.

1.4.2.1.1 Pathogens

The pea aphid remains the most serious pest of legume crops worldwide. The extensive and regular use of all categories of insecticides has induced widespread insecticide resistance and *A. pisum* has now become unmanageable by conventional insecticide use (Schmutterer 1990). These problems have provided the main impetus for development of integrated management (IPM) systems using endo-larval parasitoids or predators as key components. However, the establishment of the parasitoids in the ecosystem has been adversely affected by the extent of pesticide usage and there is a need to incorporate ecologically sound tools as alternatives to chemical insecticides. Microbial insecticides from the order of Moniliales (Fungi imperfecti) have been incorporated into the IPM system for *A. pisum* (Kogan 1998), but resistance to these biopesticides by a biotype field populations of *A pisum* has already been reported (Milner 1982) and the long term usefulness of these products must be in doubt for *A.pisum* management. Aphids are attacked by a number of natural enemies including entomopathogenic fungi, various arthropod predators and hymenopteran parasitoids. Many studies have examined the impact of these natural enemies independently of each other, demonstrating their potential under certain conditions in keeping aphid populations below damaging levels (Wilding & Perry 1980, Chambers,

Sunderland, Stacey & Wyatt 1986, Gutierrez, Hagen & Ellis 1990, Wraight, Poprawski, Meyer & Peairs 1993).

The entomophthoran fungi and their uses as biological control agents of aphids have been extensively reviewed by Milner (1997) and to a lesser degree by other workers (*e.g.* Remaudiere 1971, Hall & Papierok 1982, Verberne & Zadoks 1984, Ferron, Fragues & Riba 1986). There are comprehensive studies on biological control of pea aphid using predators, parasitoids and entomophagous fungi.

Diseases in general form an important component of the natural enemy complex of the pest. There are relatively few pathogens capable of causing high mortality in aphid populations. Among these, the fungi are by far the most important. Apparently no epizootic caused by bacteria, viruses, protozoa or nematodes have been reported for aphids (Hagen & van den Bosch 1968). In Milner (1997), the following six characteristics for ideal biopesticides were proposed: a) cheap to mass produce, b) early to sporualte, c) effective under a wide range of temperature and humidity conditions, d) provide rapid kill at economical doses, e) wide host range within aphids and f) minimal non-target effect especially on parasitoids and predators.

The aphid-attacking fungi are principally found in the order *Entomophthorales*. The genus *Entomophthora fresenius* contains most of the species that cause striking epizootic in aphid populations. About a dozen species among the 104 described within *Entomophthora* are known to attack aphids (MacLeod 1963). Hagen & Van den Bosch (1968) also reviews the impact of natural enemies of aphids.

The most common and obvious of these diseases are entomophthoran fungi such as *Erynia nepaphidis* Remaudiere & Herbert, *Entomophthora planchoniana* Cormu,

Zoophthora radicans Brefeld Barko and *Conidiobolus obscurus* (Hall & Dunn). Most of these fungal pathogens, such as *E. neoaphidis*, have a number of characteristics, which makes them successful pathogens of aphids. According to Milner (1997), these features include: i) rapid sporulation and germination, enhancing the infection process to be completed often in a few hours, ii) active discharge of conidia which maximizes the dispersal of this infectious stage, iii) high virulence whereby only a very small number of conidia is needed for infection, iv) short germination time and v) the production of resting spores in certain species, so that the fungus can persist through periods when aphids are absent or weather conditions unsuitable for transmission. In contrast, the Hyphomycete fungi (such as *Verticilium lecanii* and *Beauveria bassiana* (Balsamo)) require several days of high humidity for sporulation and infection and depend on aphid movement for contact with conidia as well as requiring a much larger number of conidia to infect. Milner (1997) therefore concludes that these fungi are much less important as natural pathogens of aphids.

Only a few pathogens have a restricted geographical range, for example *Entomophthora chromaphis* Humber & Feng has only been described from North America (Humber & Feng 1991), *Metarhizium anisoplae* Sorokin only from root aphids in the UK (Foster 1975) and *Erynia kondoiensis* Milner only in Australia and China (Milner, Teakle, Lutton & Dare 1980, Fan, Gou & Li 1991). Certain aphids seem particularly susceptible to certain fungi (Milner 1997). For example, pea aphid, cereal aphids and cabbage aphids are most frequently attacked by *E. neoaphidis*, which is the most widespread and common pathogen (Glare & Milner 1986). Other studies from tropical countries such as Chad (Silvie & Papierok 1991) and Mexico (Remaudiere & Latge 1985) have found a similar occurrence of entomophthoran fungi, as quoted by Milner (1997). Aphid-fungal pathogen associations

in South Africa are well documented. Hatting, Humber, Poprawski & Miller (1999) discovered eight species of fungi, consisting of six Entomophthorales and two Hyphomycetes, known to infect and kill the aphid host. Most of their findings were from cereal aphids. With regard to pea aphid populations in the USA, Pickering & Gutierrez (1991) reported that 24.8% were killed by *E. neoaphidis*, 5.2% by *Entomophthora planchoniana* and 0.2% by *C. obscurus*. Dunn & Wright (1955) recorded a maximum infection of 30% by *Entomophthora* in four years of sampling of pea aphids. Woronina (1965) found 0.2-0.5% aphids infected during the spring and 30-50% during the summer at Leningrad form aphids collected in the field on pea plants and assessed 2-3 days after collection. Also Angalet (1970) also assessed the degree of infection by dissecting aphids collected live from the field and found 52 - 90% of the pea aphids infected with *Erynia neoaphidis*. This result supported the notion that the fungal parasite *E. neoaphidis* was the primary cause of high mortality in *A. pisum* populations during the wet period. *Erynia* controlled *A. pisum* below economic levels only during wet periods when humidity was sufficiently high for its transmission (Milstein, Brown & Nordin 1983). Once the pathogen and aphid host is present then environmental factors, especially moisture, become critical. These fungi require very high humidity (close to 100% to sporulate, germinate and infect (Milner & Bourne 1983)). However, they are well adapted to take advantage of short periods of high humidity at night. Numerous studies have shown that aphids in moist climates, or on irrigated crops, are much more likely to be diseased than aphids on non-irrigated crops or in dry conditions (Evalkova & Voronina 1965, Frezzi 1972).

Evalkova & Voronina (1967) for example claimed that irrigation of alfalfa crops increased the proportion of fungal infection of spotted alfalfa aphid and pea aphid

respectively. Frezzi (1972) found over 80% disease presence in populations of pea aphid in irrigated fields, while in non-irrigated fields the level of disease did not exceed 2.5%. Conidia of *E. neoaphidis* germinate only at 95% relative humidity and above (Wilding 1971).

In recent years, research on mycoinsecticides has been dominated by a small number of species of Hyphomycete fungi. Two of these, *B. bassiana* and *M. anisopliae* have been used as bioinsecticides in countries such as China and Brazil for many years (Milner 1997). Now there are several products registered in the developed world. However, it is only Hyphomycetes that regularly causes mortality of aphids under natural conditions. The successful development of oil-based formulations has increased field efficacy and reduces the need for high humidity environments for germination and infection (Bateman, Carey, Moore & Prior 1993). The product Vertalec™ was first introduced for control of aphids on chrysanthemums in 1981, while another strain for control of whitefly on cucumbers and tomatoes, Mycal™, was first used in 1982 (Quinlan 1988).

Fungi generally may be more important in regulating aphid populations than is commonly thought. Species of *Pandora*, for example, have characteristics that make them good regulators of pea aphid density. They produce large numbers of infective units up to 400,000 spores from a single *A. pisum* (Milner 1981) and, depending on temperature, have a shorter generation time than its hosts. It takes as little as 2 days to complete development in *Acyrthosiphon* (Wilding 1970). *Pandorus neoaphidis* has the ability to reproduce rapidly, but must kill its host to complete development, and, given favorable conditions can regulate *A. pisum* populations. Milner (1982) recorded *Erynia* spp. as the main fungal pathogen attacking pea aphid in southeastern Australia. Even though pest populations are considerably

reduced by the natural epizootics of fungal disease, they actually contribute little to practical control. This is because the fungi are mainly effective in high-density populations when weather conditions are suitable (Milner 1997).

Van den Heuvel, Hummelen, Verbeek, Dullemans & van der Wilk (1997) isolated a new virus from the pea aphid that, under laboratory studies, reduced the growth of the aphid and increased the time needed to reach maturity. Although small isometric particles have been reported in several aphid species, only two of them RhPV and ACPV, have been partially characterized. RhPV causes a reduction in longevity and reproduction (D'Arcy, Burnett, Hewings & Goodman 1981), whereas ACPV causes aphid paralysis and rapid death (Williamson, Rybicki, Kasdorf & Von Wechmar 1988). The mode of transmission of these aphid viruses is not well understood. There are no comprehensive research results on viral biological control of aphids and therefore one has to agree that it would be premature to discuss the topic in any detail.

All authors agree that more research is required on the biology, physiology and ecology of insect-infecting fungi before the natural epizootics can be artificially induced. The scattered success of spraying fungi to control aphids certainly warrants further research. The affinity of most of these fungi for aphids makes them ideal candidates for use in integrated control programs.

The impact of natural fungal epizootic on aphid populations unquestionably can save wild and cultivated vegetation from severe general damage, but many agricultural crops may suffer considerable economic loss in spite of this type of dramatic aphid control. The aphid densities necessary to trigger fungal epizootics are often greater than the economic injury levels set for the affected crops (Bucher 1964). Pathogens that influence aphid populations

indirectly are those that weaken or cause mortality to aphid predators and parasitoid hymenopterous larvae. Parasitoid Aphidiidae larvae are killed when the aphids become infected with *Entomophthora*, since the fungi also attack the parasitoid larvae.

1.4.2.1.2 Parasitoids

The most important aphid parasitoids belong to the hymenopterous families Aphidiidae and Aphelinidae. Dipterous parasites of aphids are scarce and are only found within the Cecidomyiidae. The ichneumonoid family Aphidiidae contains most of the important parasitoids of aphids and today about 310 species are recognized in the world (Narayanan, Subba Rao & Sharma 1960, Narayanan, Subba Rao, Sharma & Starý 1962). A complete listing of the species of Aphidiidae of the world with distribution, host and bioecological data has been prepared by Makauer & Starý (1967), and that of Eastern Canada by Mackauer & Finlyson (1967). The first use of Aphidiidae in the biological control of aphids seems to have been attempted in the state of Kansas in the midwestern USA (Hagen & Van den Bosch 1968). This occurred during an outbreak of the greenbug, *Schizaphis graminum,* which was causing great losses to grain.

Solitary hymenopterous endoparasitoids of the brachonid subfamily Aphidiinae play a key role in the control of aphid populations (Starý 1970, Atanassova *et al.* 1998). Within the subfamily, the genus *Aphidius* contains the greatest number of species, including many that are used in biological control (Clausen 1977). Starý (1969) discussed the principles for use of parasitoids for biological control of aphids. Included were the geographical distribution and faunistic complexes of aphids and their aphidiine parasitoids. The taxonomy, biology, ecology and behavior of Aphidiinae are extensively covered by Starý

(1970), Unruh, Gonzalez & Woolley (1989), Starý *et al.* (1993), Pennacchio, Digilio, Tremblay & Tranfaglia (1994), Powell, Pennacchio, Poppy & Tremblay (1998).

In endemic pea aphid regions, aphidiine parasitoids and a variety of predators appear to be the most important natural enemies. In the Nearctic region, native predators often prevent pea aphid outbreaks in the spring and fall, and with the introduction of two aphidiine parasitoids, a greater degree of control has been obtained. Throughout the world, 20 aphidiiine species are recorded as parasitoids of pea aphid (Makuaer & Starý 1967).

In Pakistan, the pea aphid attacks 17 plant species and it seems to prefer pea. It only occasionally reaches damaging densities. *A. smithi* is the most important parasitoid in Pakistan but it is confined to the hilly regions, rarely extending into the plains. As a result polyphagous predatory coccinellids and syrphids were considered more important in controlling legume aphids (Ghani 1971).

Many indigenous natural enemies now exploit pea aphids in North America. In coastal California, Campbell (1926) found 20 predators, 4 parasitoids, and 2 fungi attacking pea aphids on pea. According to Smith & Hagen (1966) and Neuenschwander, Hagen & Smith (1975) parasitization by aphidiines and aphelinids during 1956-1959, prior to the spread of introduced parasitoids, was negligible. The indigenous parasitoids attacking pea aphid in California are *Aphidius ervi pulcher* Baker (= *A. pisivorus*), *Praon pequodorum* Viereck, *Monoctonus paulensis* Ashmead, *Ephedrus californicus* Baker and *Aphelinus hawardi* Delle Torre (Hagen & Schlinger 1960, Mackauer & Campbell 1972).

The introduction of the exotic pea aphid parasitoid, *A. smithi*, from India in 1958 by George Angalet has greatly changed the picture in California (Hagen, Holloway, Skiner & Finney. 1958). By 1960, this introduction had spread over much of the alfalfa-growing area

of California and had become an important controlling agent of the pea aphid in the Coastal Valley (Hagen & Schlinger 1960). It has established in eastern Oregon and Washington (Clauson 1956), and in the eastern US (Angalet & Coles 1966) from where it has spread northward into southern Ontario (Mackauer & Bisdee 1965, Harper 1976). This parasitoid has also become established in the Hawiian Island (Beardsley 1961). In the Old World, the parasite has become estasblished in Poland and Czechoslovakia by Stary (1966). The same author believes *A. smithi* to be a color variety of *A. ervi*. Releases of 100 to 300 million cage-reared *A. smithi* were made per year over a 3-year period against the pea aphid on alfalfa in Washington in order to prevent the migration of aphids from their overwintering sites in alfalfa to about 100,000 acres of peas (Halfhill & Featherston 1973).

Atanassova *et al.* (1998) reported *Aphidius ervi* as the most abundant parasitoid reared from pea aphid populations in the UK and Bulgaria. *A. eadyi* was also reared in these two countries, whilst *A. picipes* occurred within only a single pea aphid sample collected from a commercial pea crop in the UK. Of practical interest would be the examination of species that have been introduced into new geographic regions and which of these subsequently show expansions in their host range. For example, since its introduction into South America, *A. ervi* has expanded its host range to include the Russian wheat aphid, *Diuraphis noxia* Mordv., a species which it does not attack in its native area (Starý *et al.* 1993). Based on all of the recent North American surveys, Idaho is the only state where *A. smithi* is reported to attack the pea aphid (Hahn, 1983). *P. pequadorum* was reported to be the second most common primary parasitoid of the pea aphid in Iowa and Nebraska (Thiboldeaux, Hutchison & Hogg 1987).

A. ervi was introduced into North America from several European countries in 1959, and released between 1959-1963. *A. smithi* was the dominant pea aphid parasitoid up to 1968, but by the end of 1970 *A. ervi* had completely displaced *A. smithi* in the northern states and Ontario and was rapidly expanding its range westwards (Thiboldeaux *et al.* 1987). Between 1980 and 1981, *A. ervi* was released in the Australian states New South Wales (at 59 sites) and south Queensland (at 4 sites) as part of a biological control program against *A. kondoi* and *A pisum*. The parasitoid is now widely dispersed in both states (Milne 1986).

Starý (1962, 1966) believes *A. ervi* to be an effective parasitoid of pea aphid in clover or alfalfa. Firstly this is because the host is monoecious and occurring in the same habitat throughout the season, and secondly, because these crops are not too greatly distributed and are grown for more than one year. He feels that further effectiveness of *A. ervi* is due to it being oligophagous with a relatively high fecundity. The endemic parasitoids of *A. pisum* in the United States are at times important. Fluke (1926) estimated that in Wisconsin there were 11 million aphids per acre of alfalfa parasitized by *A. rosae* during one crop cutting. Campbell & Mackauer (1975) concluded from laboratory and field studies that in hot, dry areas *A. smithi* exerts the most effective control of the pea aphids whereas under mild, wet conditions *A. ervi* is predominant and has a successful controlling effect.

Species in the chalcidoid family Aphelinidae are parasitoids of Homoptera, and only *Aphelinus*, *Mesidia* and *Mesidiopsis* of the subfamily Aphidiinae are parasitoids of aphids (Hagen & van den Bosch 1968). The most well known species in the family is *Aphelinus mali*. It is nearly as well known as the vedalia beetle among entomologists (Howard 1929). This parasitoid has been introduced into the apple growing areas of 50 countries during the

last 30 years (Clauson 1956). Thus far only a very few species of the Aphelinidae have been used successfully in the biological control of aphids.

Aphelinus asychis was imported from the Middle East into California against *Therioaphis trifolii* on alfalfa (Hagen *et al.* 1958, van den Bosch 1956, Van den Bosch, Schlinger, Dietrick & Hall 1959). A strain of *A. asychis* had existed in the US prior to the importation of the Middle Eastern strain, but it only parasitized *Myzus persicae* and did not invade alfalfa to attack *T. trifolii* (Hagen & van den Bosch 1968). This same species was also introduced into Canada from Europe for biological control of the greenbug, *Schizaphis graminum* (Jackson & Eikenbary 1971).

The impact of *A. asychis* on *T. trifolli* in California was not as great as that of the two introduced aphidiids, but in the southern Californian coastal valley it predominated at times. Perhaps, a spectacular case of biological control, such as the *A. pisum* - *A. smithi* association in Hawaii, is worth mention. In this case, however, both the host and parasitoid are introduced organisms as the host plant, alfalfa, with the result that due to ecosystem conditions the alfalfa fields exhibited peculiar features. There are two basic and related questions in biological control, *i.e.* why do some introduced agents become established in a new area and others do not, and also why do some introduced agents control their target pests effectively, whilst others seem unable to do so?

DeBach (1962, 1965) examined some 200 cases in an attempt to identify the attributes of effective biological control agents. From his survey, he concluded that there was no single attribute or combination of attributes that was common to all effective agents or, for that matter, to failures. Other attempts at identifying the traits of effective natural enemies have been no more successful.

1.4.2.1.3 Predators

Aphids are attacked by a variety of predatory species that, singly or in concert, can exert a heavy toll. Aphid predators occur in many insect orders, but are mainly found within the families Coccinellidae (Coleoptera), Syrphidae and Cecidomyiidae (Diptera), Chrysopidae (Neuroptera), and Anthocoridae (Heteroptera). The complex of natural enemies on alfalfa is diverse (Pimentel & Wheeler 1973, Frazer, Gilbert, Nealis & Raworth 1981), and predators (*e.g.* coccinellids, heteropterans, lacewings, syrphids, and spiders) are considered more important than parasitoids in preventing aphid outbreaks (Frazer *et al.* 1981, Gutierrez *et al.* 1990). Of all the predators on alfalfa, coccinellids are accepted as the most important for aphid suppression, in part because of their abundance (Barney, Lamp, Armbrust & Kapusta 1984, Flint 1985) and high consumption rates (Losey 1996). The members of dipterous family, Syrphidae, are diverse in habit, which certainly holds true for the aphidophagous species. Syrphids rank as major natural enemies of aphids. Larvae are voracious feeders and often occur in great abundance in aphid colonies (Hagen & van den Bosch 1968). *Syrphus ribesii* is a common Holartic species that can devour up to 500 aphids during its larval development, whereas the larvae of S. *corollae* can consume more than 800 aphids (Sundby 1966). Within the family Chrysopidae, the Holarctic *Chrysopa carnea* is an extremely common insect, based on the abundance of the adult and egg stages, and yet its larvae are rarely found as commonly in aphid colonies as are those of coccinellids and syrphids (Hagen & van den Bosch 1968).

The predaceous coccinellids are linked to biological control more often than any other taxa of predatory organisms. The beneficial status of these organisms has a rich history that is recognized by the general public and biological control practitioners alike (Hodek 1973,

Klausnitzer & Klausnitzer 1979, Gordon 1985, New 1991, Majerus 1994). Coccinellidae are important natural enemies of pest species, especially whitefly (Gerling 1990), aphids (Hagen & van den Bosch 1968, Hodek 1973, Hagen 1974, Evans & Swallow 1993, Hodek & Honek 1996), mealybugs (Honek 1985), scales (DeBach & Rosen 1976, Drea & Gordon 1990), and mites (McMurry, Huffaker & van de Vrie 1970, Chazeau 1985). The widely cited biological control project in USA using the imported vedalia beetle (Caltagirone & Doutt 1989, DeBach & Rosen 1991) has been followed in numerous, although frequently unsuccessful programs (Hagen 1974, Gordon 1985). Similarly, many attempts have been made to encourage increased predation through augmentation, with varying results (Davidson 1924, DeBach & Hagen 1964, Hagen 1974).

Coccinellid predation has a considerable impact on aphid numbers during warm weather, but coccinellids alone are incapable of controlling pea aphid in alfalfa throughout the season (Frazer *et al.* 1981). These authors demonstrated that the density of pea aphid at Vancouver (Canada) rarely exceed 10/terminal, yet when field cages are placed over the alfalfa plants, the density increases to 250/stem or more. Pimentel & Wheeler (1973) reported 591 species in their insect surveys of alfalfa fields and of these, 216 were predators and 63 parasitoids. A 3-year study on predator abundance in two areas of California (Neuenschwander *et al.* 1975) concluded that predators were responsible for the high degree of naturally occurring biological control. Relatively few aphidophagous coccinellids have been established through importation programmes (Gordon 1985, Day, Prokrym, Ellis & Chianese 1994, Hoebeke & Wheeler 1996). *H. convergens* from California was released in South America and presumably established because of a similar geography that fitted its migratory and overwintering behaviors (Hagen, Bombosch & McMurtry 1976).

The coccinellids, *Coccinella septempuctata* L. *Hippodomia variegata* (Goeze) and *Propylea quatuordecimpunctata* L. are abundant in a wide range of Palearctic and Afrotropical agroecosystems, preying upon several economically important aphid species (Aalbersberg, Walters & van Rensburg 1984, Angood 1985, Honek 1985 [as quoted by Obrycki & Orr 1990], Chambers, Dixon, Hodek & Rabbase 1993,). These coccinellids are also established in North America (Schaefer & Dysart 1988). Currently these coccinellids are collected and reared for the biological control programme against the Russian wheat Aphid.

Coccinellids play an important role in aphid population regulation and are known to have the strongest impact of all aphidophagous insects (Hagen 1962, Hodek 1970, De Bach & Huffacor 1971, Kring, Gilstrap & Michels 1985). They sometimes played an important role in aphid regulation in field crops. A high abundance of natural enemies, especially of *Coccinella septempunctata* and *Adalia variegata*, has satisfactorily suppressed *Aphis fabae* populations below damaging levels on sugar beet in the USSR and Japan (Delucchi 1976). Sequential introduction of *C. septempunctata* and *Chrysopa cornea* resulted in 60% all-season control of aphids on potatoes in the USA (Shands & Simpson 1972). Shands, Simpson & Gordon (1972) considered *Coccinella* spp. to be more effective in controlling aphids in high-level treatments, whilst *Chrysopa* was more effective in low levels. El Hag (1992) reported that yield in caged wheat treated with mixed coccinellid species, is not significantly different from the yield of aphid-free wheat.

The impact of aphidophagous natural enemies of pea aphid in alfalfa has been evaluated using empirical studies (Neuenschwander *et al.* 1975) and simulation studies coupled with field inventories (Gutierrez, Baumgaertner & Summers 1984). These studies

suggested that predators, parasitoids and pathogens in concert could suppress pea aphid populations. Neuenschwander *et al.* (1975), Suter & Keller (1977) and Gutierrez *et al.* (1984) found that coccinellids were able to bring down pea aphid populations level in perennial crops, but that the coccinellids did not prevent the build-up of high and possibly damaging aphid populations.

Ehler (1990) and Benery & Lamp (1994) noted that, in agricultural systems, the use of natural enemy complexes, as opposed to a single enemy strategy, has been a controversial issue in the management and biological control of pests. There are cases in which enemy complexes provide enhanced pest suppression (Frazer *et al.* 1981, Mordoch 1990, Dobel & Denno 1994, Losey & Denno 1998a), but there are other instances in which predator complexes are less effective in reducing pest populations (Rosenheim, Kaya, Ehler, Morois & Jaffee 1995).

Several non-prey specific predators (generalized), including aphidophagous coccinellids used in classical biological control have not been easily established or did not result in successful biological control (Hagen 1962, Gordon 1985). Various reasons have been proposed to explain these poor results, including lack of understanding of prey-specificity and potential competition for prey with existing indigenous predatory species (Thompson 1951, Hagen *et al.* 1976). For example, release of *C. septempuctata* in the US occurred over a 15-year period with little apparent success. Ladybeetles have been successfully used for the biological control of coccid pests (Hodek 1973, Bartlett 1978), whereas their role as biological control agents of aphids, in most cases, has been disappointing. The efficacy of the predaceous coccinellids in natural or managed systems is difficult to determine, given their mobility and typical polyphagous nature (Cooke 1963).

1.4.2.2 Biological control of fungal pathogens

If we limit the concept of biological control as the one-on-one use of introduced organisms to control pests and diseases, then its contributions have been modestly significant for insect pests and weeds, but of minor or no significance for plant parasitic nematodes and plant diseases (Cook 2000). Thousands of species of natural enemies of insect pests were introduced and released for the worldwide biological control of insect pests during the twentieth century. Indeed, the foundation to this approach of biological control of insect pests was already in place at the beginning of the twentieth century, with the successful control of certain pests.

Most biological control strategies are directed toward soilborne plant diseases and involve the use of antagonist (fungi, bacteria, and nematodes). The search for biological control agents against pathogens causing quiescent infections (*e.g.* ascochyta) has been more difficult because the infecting hyphae are protected from microorganisms once the pathogen has penetrated the plant cuticle (Lucas 1997).

Nematodes are the most abundant soil-inhabiting animals. Fungivorous nematodes feed on mycelia of many different species of soil fungi, including beneficial fungi and plant pathogens (Faulkner & Darling 1961, Barnes, Russell, Foster & McNew 1981). Numerous species of fungivores have been found in soils (Nicholas 1984). The most common genera found in agricultural soils are *Aphelenchoides*, *Aphelenchus*, *Tylenchus*, and *Ditylenchus* (Hofman & Jacob 1989, Yeates, Wardle & Watson 1999) and some species within these genera are plant parasites.

Isolates of the soil fungus *Trichoderma harzianum* Rifai have been described as a potential biological control agent against several soilborne plant pathogens (Knudsen,

Eschen, Dandurand & Wang 1991). The soil inhabiting ascomycetes *Talaromyces flavus* (Klöcker) suppresses verticillium wilt of tomato, eggplant and potato (Dutta 1981, Marios, Johnston, Dunn & Papavizas 1982) and parasitizes *Sclerotinia sclerotiorum* (McLaren, Huang & Rimmer 1986) *Rhizoctonia soloni* (Boosalis 1956) and *S. rolfsii* (Madi, Katan & Henis 1992). In general the mechanisms implicated in antagonism toward biological control of phytopathogenic fungi include mycoparasitism, antibiosis, competition and induced systemic resistance (Elad, Chet & Henis 1982). Cell wall degrading enzymes, such as β-1, 3-glucanase, cellulase, and chitinase are involved in the antagonism activity of biological control agents against phytopathogenic fungi (Chernin, Ismailov, Haran & Chet 1995, Haran, Schickler, Oppenheim & Chet 1996). In addition to fungi and nematodes, bacteria of the genus *Streptomyces* and *Pseudomonas* have been shown to parasitize and/or inhibit *Phythium* sp., a pathogenic fungus.

Seed dressing with microbiological material prepared from *Tylenchus koninhii* and *Gliocladium rosuem* were found protecting pea from *A. pisi, Botrytis cinerea, Rhizoctonia solani* and *Fusarium* spp. found in the soil (Lacicowa & Pieta 1996). The bacterium, *Bacillus cereus*, *Enterobacter agglomeranse* and *Klebsiella ozaenae* reported inhibiting the growth of *Ascochyta rabiei* in laboratory tests (Shahid & Riazuddin 1999). As with all other control tactics in the IPM arsenal, biological control of pests of crops shows the best results when it is a component, preferably the key component, of the IPM strategy. Biological control, when fully integrated with all other control tactics, including the conservative use of selective pesticides, when needed, increase the cost-effectiveness of the pest control system.

1.4.3 Cultural control

Cultural practices employed within crop habitats can affect pest species and biological control agents directly by minimizing crop damage or indirectly by altering microclimatic conditions within the crop habitat. Activities that produce such microenvironment modifications include irrigation, fertilizer application, row spacing, seeding rate, planting time, intercropping and tillage. These practices may lead to substantial improvement in benefits of biological control, especially in cases where changes are more favorable towards natural enemy populations than to pest populations.

Multiple cropping systems have been suggested as a possible mechanism for the management of pest species. Vandermeer (1989) discussed several types of multiple cropping systems and defined intercropping as the simultaneous cultivation of two or more crops in the same field. Although many intercropping systems may have evolved to take advantage of a beneficial plant-to-plant interaction (e.g. nitrogen fixation), some intercropping systems are being investigated and recommended for the reduction of insect pest load (Risch, Andow & Altieri 1983). However, results from studies attempting to verify the benefits or otherwise of such practices have been mixed (Seehan 1986) and demonstrate the importance of understanding the nature of multitrophic interactions. Probably the best known example of the benefits of mixed cropping is the re-diversification of the cotton agroecosystem in the Canete Valley in Peru, after massive bollworm outbreaks led to the abandonment of mixed cropping in the 1950s (Dout & Smith 1971, as cited in Verkerk, Leather & Wright 1998).

Intercropping may increase the effects of natural enemies because one of the intercropped plants provides the allelochemical attraction or a nectar source for natural

enemies, or because the intercrop improves conditions (*e.g.* moisture, shelter) for ground-dwelling predators (van Emden 1989). Read, Feeny & Root (1970) suggested that planting collards near beet may inhance biological control of beet pests, since collards attract braconid parasitoids (*e.g. Diaeretiella rapae* M'Intosh) to the general area. Field studies in Mexico showed that parasitism of *Diaphania hyalinata* L. was greater in tricultures (squash, maize, legumes) than in monoculture of squash (Letourneau 1987), providing partial support for the enemy hypothesis (Root 1973) which dictates that natural enemies should be more abundant in diverse rather than in simple habitats. However, the author also found that parasitoid attack was elevated in maize monoculture and that predator abundance was not enhanced in the tricultures, with some predator species actually being more abundant in the monocultures. In a study of maize monocrop and maize-cowpea intercrop field experiments in Kenya, Päts, Ekbom & Skovgärd (1997) found no change in oviposition behavior of the herbivore pests (*Chilo* spp.), but did find increased egg parasitism in the intercrops. Abundance of an important ground-dwelling predator, *Pterostichus melanarius* Illiger, was found to be greater in barley-pea intercrop than in monocultures of barley, pea, faba bean or fescue (*Festuca rubral*). These authors attributed the preference shown by the beetles to both visual and chemical cues from the intercrop.

Mixed cropping has been reported to promote natural biological control and reduce pest populations in certain crop combinations (Matteson, Altieri & Gagne 1984, Altieri & Liebman 1986). The practice has been shown to reduce thrips populations in different crops (*e.g.* cowpea/sorghum) (Matteson 1982, Ezueh & Taylor 1984, Alghali 1993, Kyamanywa, Baliddawa & Ampofo 1993, Kyamanywa, Baliddawa & Omolo 1993). Weiss, Schatz, Gardner & Nead (1994) demonstrated that flea beetle counts were highest in oilseed rape

monoculture compared with those intercropped with field pea, but this was not significant (except for one year). The land equivalent ratio did also not exceed 1.0, indicating that the intercrops selected were not as efficient in yield as the monoculture. They concluded that the intercrop system was not effective in reducing flea beetle loads on a per-plant basis, nor was there a yield advantage from this intercrop system. A similar result was reported by Bottenberg, Tamo & Singh (1998), which indicated that mixed cropping had little effect on cowpea pests and natural enemies, confirming earlier studies on cowpea-sorghum and cowpea-maize intercropping from Nigeria (Matteson 1982, Fischer, Raheja & Elemo 1987). However, according to Singh & Kothari (1997), intercropping of mustard with fennel (an aromatic plant) resulted in a significantly lower aphid (*Lipaphis erysimi* Kaltenback) infestation. Gorfu (1999) noted that there was noticeable influence of mixed cropping (faba bean and field pea) on disease (Chocolate spot and *Ascochyta* blight) development and yield. The land equivalent ratio exceeded one for mixed crops, showing that the productivity of mixed cropping of the two species was superior over pure culture of each.

Field trials have also been conducted to manage various aphid species by intercropping. These included cotton aphid (*Aphis gossypii*) (Bai & Zhou 1982, Vieira, Santos Das & de Oliveira 1983, Potts & Guandi 1991), potato aphid (*Macrosipum euphorbiae*) and green beach aphid (*Myzus persicae*) (Mckinlay 1985, Afili, Haydar, & Omar 1990, Potts & Guandi 1991, as quoted by Singh & Kothari 1997). In central Mexico, two *Hippodamia* species were more prevalent in maize-faba bean polycultures than in maize monocultures, presumably because of the availability of extrafloral nectaries in faba beans (Altieri 1994). Conversely, in the United States, *C. maculata* was more common and consumed more European corn borer in maize monoculture than in two polycultures, a fact

attributed to increased aphid densities and spatially distributed maize pollen in the monoculture (Andow & Risch 1985).

Most studies on field pea intercropping on diseases have used faba bean. In fact faba bean and field pea are frequently mixed in Ethiopia where most field pea is produced (Ghizaw, Fissahaie & Alemayehu 1993, IAR 1993). Studies have been conducted under both temperate and tropical conditions and for different cropping patterns. However, the effects of mixed cropping on pea aphid and their natural enemies are not understood in the Ethiopian context.

Kennedy (1958), in reviewing the effects of physiological variation in plants on their susceptibility to aphids in terms of host selection, plant growth and senescence, plant water relations and varietal resistance, stressed the importance of the quality of food available to the aphids, and particularly of the soluble organic nitrogen content in the sieve-tube sap. El-Tigani (1962) has reviewed the results of many authors on the effects of most of the major plant nutrients on several species of aphids. He also conducted a large series of experiments using several aphid species on several host plants supplied with varying amounts of nitrogen, phosphorous, potassium and calcium. He concluded that most deviations from the normal mineral fertilization increased the susceptibility of plants to aphids, as quoted by van Emden (1966). Van Emden & Wearing (1965) proposed that partial host plant resistance could be induced by altering the physiological status of plants via a fertilizer regime (*e.g.* different levels of nitrogen and potassium) and degree of water stress. They showed that the abundance two aphid species, *B. brassicae* and *Myzus persicae* (Sulzer), could be depressed on brussels sprouts by a high potassium/good irrigation regime and that such 'cultural'

resistance would be compatible with the action of natural enemies under field conditions (van Emden & Wearing 1965).

Habitat or environmental manipulation has proved to be another form of conservation and augmentation of natural enemies. Here the cropping system is successfully altered to augment and enhance the effectiveness of natural enemies. It has been observed that adult parasitoids and predators have significantly benefited by the source of nectar and the protection provided by refuge. In the conservation of the natural enemies, Starý (1970) used the term 'parasite foci' with reference to refugia for parasitoids of aphids and stressed that foci existing inside the given crop, in allied crops and in neighboring areas such as uncultivated land and hedgerows must be distinguished if conservation is to be attempted.

According to Landis, Wratten & Gurr (2000) conservation of biological control agents involves the manipulation of the environment to enhance the survival, fecundity, longevity, and behaviour of natural enemies to increase their effectiveness. For example, maintaining the presence of the weed *Cytisus scaparius* (Broom) in central Europe has been shown to be beneficial since it helps in maintaining the parasitoid *A. ervi* on economically unimportant aphid hosts (Starý 1970). The resulting increased abundance of *A. ervi,* in turn, assists in regulating the economically important aphid, *A. pisum*, on alfalfa in the same region. Cowgill, Wratten & Sotherton (1993) also demonstrated an increase in syrphid populations through the selective use of floral resources.

Adjusting planting time to escape pest damage is the most important means of keeping pest damage below economic level. For example, early planting is perhaps the most effective means of control against stemborers on sorghum and maize in many parts of Africa and is widely practiced by farmers (Abate & Worku 1998, Gebre-Amlak, Sigvald &

Pettersson. 1989, Ebenebe, van den Berg & van der Linde 1999). Early sowing is a means of reducing damage of field pea by pea aphid (IAR 1998). In Ethiopia, the time of sowing of field pea is determined by the degree of importance which the farmer attaches to the crop. Normally, farmers sow their main food crops, *e.g.* cereals, first, after which subsidiary crops such as legumes are planted.

For some pests, population dynamics vary between seasons, as well as within a season, and time of sowing is used to avoid peaks of pest populations at the critical stage of the crop (Nderitu, Kayumbo & Mueke 1990, Smit & Matengo 1995). Farmers are often aware of such pest population fluctuations and their effects on crop performance and therefore adjust their planting dates accordingly. For example, sweet potato planting is delayed to avoid sweet potato weevil (*Cylas* spp.) damage in western Kenya (Smit & Matengo 1995).

Time of sowing can have a significant effect on the severity of the fungal disease *Mycophaerella pinodes*. Barbetti & Brown (1993) showed that early sowing produces severe *M. pinodes* infections through exposing the field pea seedlings to heavy releases of airborne spores that occur in autumn. Delaying sowing generally reduces the severity of *M. pinodes*. In a sowing date trial at Holetta (Ethiopia), field pea sown during the first week of July resulted in a significant reduction of *M. pinodes* incidence (IAR 1997). In western Australia, Barbetti & Brown (1993) also indicated that a two-year break between field pea crop cultivation significantly reduced the level of disease infection. The use of high quality seed (with low level of *M. pinodes* infection) is important, since infected seeds could carry the disease to new areas and act as primary sources of infection for initiating the disease under favourable environmental conditions. Seeds from low rainfall areas are likely to carry less

ascochyta infection than seeds from high rainfall areas, because leaf wetness is one of the major factors affecting disease and fruit body development for ascochyta blight (Huber & Gillespie 1992. Crop rotation, using a diversity of broad-leaf and cereal crops, will also ensure lower levels of ascochyta blight (Barbetti & Brown 1993).

1.4.4 Chemical control

Synthetic pesticides have been used for many years to control pea aphid. There is a voluminous array of reports on chemical control of pea aphid in peas and alfalfa. A wide range of chemical insecticides has been tested, most frequently as liquid formulations. All of the insecticides that have been evaluated have been found to reduce grain damage and/or increase yields relative to untreated control plots in one or more studies (*e.g.* Stark & Rangus 1994, Biddle, Blood-Smyth, Tabot & Smyth 1994, Bhatnagar 1996). The first insecticides were tested in the early 1960s and included DDT, parathion, dieldrin and methyl demeton. More recently organophosphate, carbamate and synthetic pyrethriod insecticides have been evaluated for pea aphid control. The insecticides most frequently reported as providing effective control against pea aphid on peas are malathion, dimethoate, diazinon, pirimicarb, primiphos-methyl, chloropyrifos, endosulfan, carbofuran, and demeton-s-methyl (Mueke, Manglitz & Kehr 1978, Maitaki & Lamb 1985, Yencho *et al.* 1986, Stark & Rangus 1994, Biddle *et al.* 1994, Bhatnagar 1996, Ali 1997). Many of the insecticides commonly used today are devastating to populations of biological control organisms (Croft 1990) and pollinators (Johanson 1977) in that they hinder the implementation and effectiveness of IPM (Poehling 1989). One direction in chemical insect control that might alleviate this situation is the development and use of selective insecticides. Such chemicals may offer an opportunity for integration of chemical and biological control in a more sustainable way.

Natural plant products and their analogues are an important source of new agricultural chemicals (Gulter 1998) used in the control of insect pests (Emosairue & Ukeh 1996) and plant diseases (Amadioha 1998, 2000). Stoll (1986) lists a number of plant species with insecticidal properties, as well as guidelines for the extraction of the active ingredients.

Pesticides derived from the neem tree, *Azadirachta indica* A. Juss, appear to be promising pesticides for use in IPM programs for some pest species. Neem pesticides are reported to provide broad-spectrum control of more than 200 insect species (Ascher 1993), yet remain compatible with beneficial species (Schumutterer 1990). It is also effective against a wide variety of other organisms (Saxena 1989) and shows a great potential as an important source of botanical insecticides. It has low mammalian toxicity (Jacobson 1988), degrades rapidly in the environment, is relatively non-toxic to many natural enemies (Schmutterer 1990, Markandeya & Divakar 1999), has no known mutagenic effects and the development of resistance appears to be relatively slow (Saxena 1989, Schmutterer 1990). Neem insecticides are, therefore, candidates for use in the control of pea aphid, the major pest of commercially grown peas in western Washington (Stark & Rangus 1994). Recent studies support the use of neem insecticides to control aphids (Lowrey, Isman & Brard 1993, Lowrey & Isman 1993, 1996).

Several formulations of neem extracts have been evaluated for control of pea aphid. Schauer (1987) showed that various crude neem seed kernel extracts were toxic to *A. pisum* and *Aphis fabae* Scop. The study of Lowrey *et al.* (1993) indicated that neem insecticides provided adequate control of several aphid species in the field. A 10 % seed kernel extract (Ali 1995) resulted in less pea aphid damage in the field. Research conducted at the International Center of Insect Physiology and Ecology (ICIPE) in 1992 showed that spray

application of 3 % aqueous neem seed extract on cowpea controlled thrips and grain yield obtained was equal to that in plots sprayed with cypermethrin (0.04 kg a.i/ha). A more recent study of Musabyimana, Saxena, Kairu, Ogoi & Khan (2001) has also reported the repellent and feeding deterent effect of neem materials against banana corm borer, *Cosmopolites sordidus* (Germar).

Low doses of a neem extract and azadirachtin (a primary active ingredient of neem) on plants have been shown to reduce the fecundity of several aphid species (Lowrey & Isman 1994). It has also been suggested that sub-lethal doses of azadirachtin may make a significant contribution to the control of the peach-potato aphid, *M. persicae*, while having little or no adverse impact on aphid parasitism by *Aphidius matricariae* Haliday (Sugden 1994). Many commercial formulations of azadiractin are now available and this has increased interest in using neem to control pea aphid. Neem insecticide formulation Margosan O has been reported to reduce the number of molts, longevity and fecundity of *A. pisum* reared on treated *Vicia faba* plants (Stark & Rangus 1994).

Control measures aimed at limiting the development of ascochyta blight are based on repeated protectant sprays of fungicides applied at 10-15 day intervals from flowering to pre-harvest, regardless of the risk of infection (Roger *et al.* 1999). Seed treatment effectively reduces seed borne Ascochyta to nondamaging levels. Seed dressing fungicides like Pickel T® [at] a rate of 150ml/100kg seed provide good insurance against early infection and improve vigour (Bbarbetti & Brown 1993). Foliar sprays of LB-Pickel (carbendazim 26%, vinclozolin 30% and thiram 34%) at a rate of 1 kg product in 250 l water/ha was noted to control ascochyta blight by fortnightly application in Australia (Bretag *et al.* 1995). In France a mixture of flutriafol and chlorothalonil was also used to control the disease during periods of

disease risk (Tivoli *et al.* 1996). In the fungicide screening trial at Holetta (Ethiopia) against foliar diseases of field pea, Bravo – 500, Benlate, Thiophanylmethyl and Redomyl were effective and increased yield and 1000-seed weight significantly (IAR 1997).

1.5 Synthesis

Insect pests and diseases are a major constraint to field pea production, yet there has been relatively little research investment, particularly outside of the developed countries, into the biology, ecology and management of the pests and their natural enemies. To some extent research has concentrated on host plant resistance and biological control. Knowledge of the impact, dynamics and ecology of the pest and its natural enemies is essential before effective control strategies can be developed. It should be stressed that understanding plant-pest-natural enemy interactions is essential to the successful integration of plant resistance with biological control for optimal IPM results. These studies must focus on the cropping systems, as field pea is frequently one component of a complex farming system. There is no short cut to reduce losses due to pests immediately. Progress will be incremental and in the short term, the greatest impact may come from improving pesticide application and developing safe alternatives that have potential to replace the toxic pesticides. The incompatibility between chemical and biological control has in fact been the main force behind the evolution of IPM. Strategy for medium term should concentrate on developing improved cultivars that combine high yield, disease and insect-resistance with good agronomic characters. A longer-term solution to insect pest problems in field pea must focus on ways to enhance natural control processes by enhancing the effectiveness of endemic species. Exploitation of underutilized natural enemies, development of novel bio-pesticides and management of resistance are all tactical options to enrich IPM strategies. All the

aforementioned control tactics yield the best results when they are a component of the IPM strategy. IPM should be given the highest priority as a pest management strategy for developing countries. How it should be developed accentuates the need for focussed research on all the components of IPM.

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Table 1.1 Mean area, production, and yield of dry peas in various regions and countries in the world in 1998.

Region/country	Area (1000 ha)	Production (1000 MT)	Yield (kg/ha)
Africa	583	346	651
Ethiopia	180	160	889
Congo DR	110	68	618
Tanzania	60	22	367
Morocco	50	15	300
Burundi	54	33	611
North & central America	968	2067	2136
Canada	848	1762	2079
USA	115	300	2608
South America	74	78	1048
Peru	30	26	865
Argentina	20	30	1500
Ecuador	12	9	794
Asia	1508	1997	1324
China	700	1250	1786
India	580	600	1018
Pakistan	142	79	551
Nyammar	39	28	714
Bangladesh	18	14	775
Europe	3041	7753	3025
France	625	3200	5120
UK	78	297	3793
Denmark	69	257	3708
Hungary	52	101	1938
Austria	31	93	3010
Poland	25	55	2233
Rumania	22	27	1241
Oceania	287	50	1086
Australia	321	299	931
New Zealand	16	51	3117
World	6486	12591	1949

Source: FAO 1999.

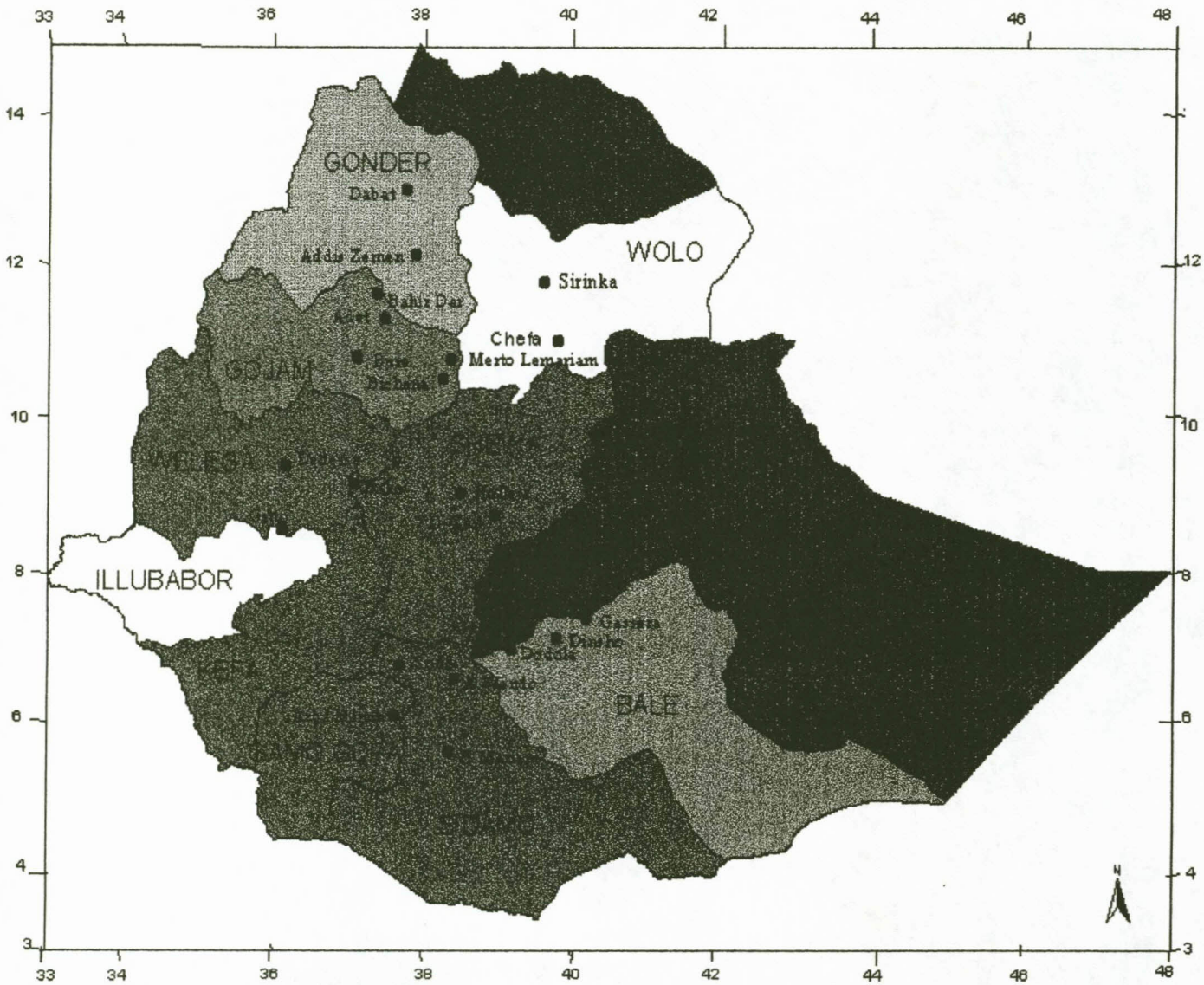
Table 1.2 Means of total area cultivated, total production and grain yield of major pulse crops for private peasant holdings in Ethiopia for the period 1997/98 and 1999/2000.

Legumes	**Area ('000 ha)**	**Production ('000 tonnes)**	**Yield (kg/ha)**
Faba bean	319.50	330.54	1027
Chickpea	176.80	166.72	854
Field pea	141.55	105.81	747
Haricot bean	138.43	105.78	746
Lentil	59.86	39.75	657
Vetch	114.59	99.58	877
Total	949.73	832.76	

Source: CSA, Central Statistical Authority (2000).

Note: Figures for production and yield do not account for the substantial amount of green seeds of faba bean, field pea and chickpea eaten in the field.

Figure 1.1 Map showing the distribution of *Acyrthosiphon pisum*



CHAPTER 2

Sources of resistance in field pea, *Pisum sativum* L, to two strains of pea aphid, *Acyrthosiphon pisum* (Harris)

Abstract

Resistance to two strains of the pea aphid, *Acyrthosiphon pisum* (Harris), was assessed in 30 genotypes of field pea, *Pisum sativum* L. Population density ratings based on the number of aphids per plant and seedling mortality showed that six lines had the highest level of resistance to the South African *A. pisum* strain, while three lines were moderately resistant to the Ethiopian population.

It is clear from the results of this study that there were differences between the two strains of pea aphid in the lines evaluated, regarding their survival and reproduction. These results reiterate the need to take into account aphid genetic diversity in breeding programs for resistance in peas.

Key words: *Acyrthosiphon pisum*, *Pisum sativum*, host plant resistance, South Africa, Ethiopia.

Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris), is an insect pest of considerable economic importance in the production of legume crops, particularly peas, *Pisum sativum* L., alfalfa, *Medicago sativa* (L.), clover, *Tripholium partense* (L.) and lentils, *Lens culinaris* Medikus (Forbes & Frazer 1973, Maiteki, Lamb & Ali-Khan 1986). In Ethiopia, the pest was first recorded in 1947 (cited as *Macrosiphum pisi*) (Crowe & Ali 1983) and has since spread throughout field pea and lentil producing areas to become the most important pest of these crops. The status of pea aphid in southern Africa is not clear and Daiber (1994) did not include it as a pest of peas and green beans. Feeding on leaves and developing pods by pea aphids depletes plant nutrients and causes reduced plant vigour, poor seed quality and low yields. Ali (1997) reported a yield loss of 49% in untreated field pea fields in Ethiopia. Insecticides that provide effective pea aphid control have been identified and optimal application times determined (Ali 1999). However, most farmers in Ethiopia are resource-poor and cannot afford the use of insecticides. In a recent survey carried out in the country, <1% of the farmers use pesticides (Abate 1997). Moreover, yields of field pea grown under traditional farming systems are generally low and this suggests that other aspects of control will assume increasing importance in the future.

In crops with a narrow profit margin, such as field pea, plant resistance to *A. pisum* may be an especially important control measure. It is the least expensive method of crop protection and perhaps the only one available to resource-poor, small-scale farmers in the developing world. It is therefore important to develop varieties that possess a high level of resistance to pea aphid. Not only could the use of resistant field pea varieties reduce economic loss from *A. pisum*, but may also reduce economic costs and environmental

damage associated with the use of aphicides to control the pest. However, relatively few studies have evaluated and identified resistance to *A. pisum* in field pea accessions (Markkula & Roukka 1971, Newman & Pimentel 1974, Bieri, Baumgartener, Bianchi, Delucchi & von Arx 1983, Holt & Wratten 1986, Mackay & Lamb 1988, Soroka & Mackay 1991, Holtkamp & Clift 1993, Downes 1994).

Biotypic variation is of major concern when developing plant resistance. In this study a population of an insect species that can inflict damage to plant entries in a certain area, normally resistant to that insect are referred to as a 'biotype' (Puterka & Burton 1990). This phenomenon commonly occurs in aphids targeted for management with plant resistance (Via 1991, Sandström 1994, Bournoville, Simon, Badenhausser, Girousse, Guilloux & André 2000). This experiment was conducted to detect the presence of resistance in field pea lines to a pea aphid strain from South Africa and Ethiopia, based on aphid population densities and plant survival.

Materials and Methods

South Africa. Colonies of the pea aphid, *Acyrthosiphon pisum* (Harris), were initiated from field-collected apterous viviparae from pea plants on the west campus of the Free State University, Bloemfontein, South Africa in January 1999. The aphids were reared at 20^0C and 70% RH on the field pea variety Mohanderefer that was grown in pots (15 cm diameter) filled with a standardized soil/compost mixture (1:1 soil-peat volume/volume). Incandescent light was provided for 16 hours per day, supplying adequate illumination for the healthy growth of the young plants. Plants and aphids were enclosed within organdy sleeve cages (20 cm in diameter and 80 cm in height) constructed from fine nylon mesh.

A total of 30 field pea lines and commercial cultivars obtained from Ethiopia and South Africa (Table 2.1) were screened for resistance to pea aphid. The study was carried out in a greenhouse on the campus of the University of the Free State at 20^0C, a light:dark photoperiod of 16h:8h and 60% - 70% RH. Day length was maintained at 16h by using supplementary incandescent lighting. The field pea lines were arranged in a randomized complete block design, which was replicated 3 times. Three seeds of each entry were planted in a standard greenhouse soil mixture in plastic pots (15 cm diameter) and thinned to one seedling per pot once seedlings were established. Individual seedlings were infested with four 4^{th} instar nymphs or newly emerged adults (ca. 24h old) from the culture, using a fine camel hair brush. This was done two weeks after planting, *i.e.* growth stage 102 -103 (Knott 1987). The plants were covered with organdy bags constructed from fine nylon mesh. The cages were supported over and around the potted plants by stainless steel stakes and held in place by rubber bands at the bottom to prevent aphid escape.

Seven, 14 and 21 days after infestation, aphid populations on each entry were visually scored. A modified six point rating score (Ellsbury, Pant & Knight 1985) was used, whereby 1 = no aphids, 2 = 1 - 10 aphids (adults and nymphs), 3 = 11-20 aphids, 4 = 21 - 50 aphids, 5 = 51 - 100 aphids in large colonies and 6 = >100 aphids when the entire plant is heavily infested. Plants in category 1-2 show no obvious aphid injury, plants are green and healthy; 3-4, somewhat stunted with leaves smaller and lighter green than in class 1-2; 5 extreme stunting with very small, light green or yellowish leaves, but still alive; 6 plants are dead. During scoring, all plant parts were carefully inspected for adults and immatures on each plant entry.

Seedling survival was taken as one of the criteria of resistance to Ethiopian pea aphids. Plants that were healthy and green (no stunting or yellowing of the leaves), with no apparent aphid damage were considered resistant and selected for further studies. All extremely susceptible seedlings had died during this period due to severe aphid attack.

Ethiopia. The pea aphid culture used was collected from the Holetta Agricultural Research Centre and continuously maintained on Mohanderfer field pea in the same environment as the one in which the study was conducted. These 30 field pea lines (Table 2.1) used in South Africa were screened for resistance to pea aphid in an insectary under natural photoperiods, mean temperature of 22.7^{0}C (day) and 15.5^{0}C (night) and 70% – 94% RH from July to September. Three seeds were planted in plastic pots (20 cm diameter) and thinned to one seedling per pot once seedlings were established. The potting medium contained sterilized soil with 250 mg Diammonium phosphate (18 N, 46 P_2O_5) per kg of soil (T. Bekele, Holetta Agricultural Research Centre. pers. comm.). Plants were watered when the soil was dry to touch. Three replications (pots) per entry were used. Individual seedlings were infested as described in section 2.2.1. The plants and aphids were then covered with clear plastic cylindrical cages (60 cm in height and 15 cm in diameter) with a cloth lid and six cloth-covered ventilation holes on the sides. Similar procedures were followed for scoring the entries on a scale of 1-6 as described in section 2.2.1

Scores were analyzed using analysis of variance (ANOVA) and means were separated with least significant difference (LSD) at $p = 0.05$ (MSTAT-C). After this preliminary study, entries with a 1 - 3 rating were selected to be evaluated in more detail and to ascertain antibiosis, antixenosis and tolerance. These are the factors that may be the underlying basis of resistance, as originally defined by Painter (1951).

Results

South Africa. Mean scores for *A. pisum* on 30 pea genotypes are given in Table 2.2, with the significance grouping from LSD. At all scoring dates there were significant differences ($P < 0.05$) in scores of pea aphid population densities among the genotypes tested (Table 2.2). Mean scores across all scoring dates ranged from 2.1 for line 30 to 4.3 for line 22. Pea aphid population level scores of the breeding lines (13 - 30) ranged from 2.1 to 4.3, whereas scores for released varieties resulting from several years of selection, ranged from 3.1 to 4.0. The rankings of the 30 lines are very similar, however, with minor differences evident. For the South African strain there is three distinct groupings, *i.e.* an extremely susceptible group (4 genotypes), a group with intermediate resistance (20 genotypes) and a resistant group (6 genotypes).

Lines 05, 19, 24, 27, 29 and 30 sustained little aphid damage, whereas line 22 and 25 were severely damaged by pea aphid feeding. These were the only two lines with seedling mortality and mean rating scores exceeding 4. Six resistant lines, i.e. Holetta Local-90, 305ps210689, 061K-2P-2/9/2, 061K-2P-14/7/1, JI 898, and 304WA1101937 and one susceptible line, NEP874UK, were selected from the screening tests and subjected to more intensive tests to ascertain their resistance traits.

Ethiopia. Genotypes differed significantly ($P < 0.05$) in terms of pea aphid incidence at individual scoring dates, and averaged overall scoring dates (Table 2.3). Pea aphid population level scores of the breeding lines (13 - 30) were very low for the Ethiopian strain, ranging from 3.4 to 5.2, whereas for the released (commercial) varieties from breeding program ranged from 4.4 to 5.9. In this initial screening 19 of the 30 field pea lines infested with pea aphid were killed or had scores of more than 4.5 three weeks after infestation. Of

the remaining lines, only 3 survived and were considered resistant. Intermediate levels of resistance (population scores ranging from 3 to 4) were found in one of the 30 lines tested. By the third week, most of the plants had scores of 6 and were thus all within the susceptible range, indicating that the length of time of the test was sufficient to detect damage. The mean population score for line 29 was less than the scores of all other entries. Four entries (line 7, 11, 22 and 25) had 100% seedling mortality resulting from pea aphid feeding while the seedlings of line 05, 27 and 29 were not damaged after exposing them for three weeks.

Discussion

The main purpose of the preliminary screening trial was to confirm field resistance and to identify a number of pea lines differing as much as possible in terms of their quality as host plants, which could then be subjected to more detailed study. In general, the pea entries performed very well against the South African *A. pisum* strain, compared to the Ethiopian one. Mean pea aphid density ratings were consistently greater for the Ethiopian strain, than for the South African strain. The Ethiopian strain displayed increased virulence on all pea lines with a mean population level score of greater than 3. However, strain variation was most apparent in the performance of field pea lines 19, 24 and 30. The seedlings of these lines scored >3 for Ethiopian strain indicating that aphid populations were higher and 66% of the plants were dead three weeks after infestation whereas these same lines had score of less than 3 (resistant) with no seedling mortality to the South African strain.

In Ethiopia, only 3 lines (05, 27 and 29) survived (*i.e.* zero seedling mortality) three weeks after infestation compared to 28 lines in South Africa. Pea aphid population levels also reflected a higher figure in Ethiopia on all pea entries tested (range 3.4 to 5.9) compared to South Africa (range 2.1 to 4.3). In this context lines 19, 24 and 30 were resistant to the

South African strain, while the Ethiopian strain inflicted serious damage with 66% of seedling mortality. Furthermore, none of the 30 genotypes investigated in this study showed a high level of resistance to the Ethiopian pea aphid strain. The fact that ranking tended to be strain specific, is demonstrated in terms of the association between the pea aphid strain and field pea genotype variables. Such plant genotype – aphid genotype interactions have been reported earlier by Harrington (1945) for pea aphid on peas in USA. However, the possibility of different aphid biotypes in South Africa and Ethiopia should not be ignored.

Entries that had performed the best across both strains were lines 05, 27, and 29. Three pea lines, i.e. 19, 24 and 30, were first identified as resistant to *A. pisum* in South Africa, but were verified as susceptible to the Ethiopian strain. The high degree of diversity found within the two *A. pisum* strains indicates that the utility of resistant plant germplasm from one geographical location to another is limited because of the possible strain variability. In a positive sense, this indicates that pea entries that are susceptible to *A. pisum* in one geographic area might actually be resistant in another geographic region, implying that breeding for *A. pisum* resistance may not be a necessity in certain geographic regions. In such areas all that may be needed is to identify those pea cultivars that may already possess levels of resistance to endemic *A. pisum* populations. In doing this, the need for an expensive breeding program could be circumvented unless populations in nature are mixed.

A high level of genetic variability may be found within aphid populations, even when reproduction is asexual (Simon, Baumann, Sunnucks, Hebert, Pierre, LeGallic & Dedryver 1999). Mackay & Lamb (1988) observed that genetic variation occurred in two species of aphids, *i.e. A. pisum* and *A. kondoi*, developing on alfalfa in Australia where asexually reproductive lines have been introduced. By measuring responses on 3 alfalfa cultivars,

these authors determined that 12 lines of *A. kondoi* were probably made up of three to seven clones, and three or four clones among 12 lines of *A. pisum.*

Markkula & Roukka (1971) reported that there was different *A. pisum* biotypes reacting differently to 103 varieties of peas tested. On the other hand, Sandström (1994) reported that pea aphid clones showed little difference among the cultivars he evaluated. Earlier studies of MŰller (1962) showed that sexual reproduction has been implicated as the primary mechanism for generating aphid biotypic diversity to resistant pea entries. *A. pisum* has anholocyclic reproduction in both countries and sexual forms have not been documented. Between the two strains used in this study, genetic variability was found within geographical regions since behavioural response of the two pea aphid strains differ on the same field pea lines. There is also no evidence that *A. pisum* in Ethiopia has differences in virulence. Results from this study suggest that *A. pisum* populations should be continuously monitored for biotypic variation in those areas where resistant plants are to be deployed, thereby ensuring that biotype-specific resistance is pro-actively identified.

It is concluded that the field pea varieties currently cultivated in Ethiopia and their different breeding lines are moderately resistant to susceptible (4 – 5 scale) to *A. pisum* damage. As such, control of this pest will have to involve the use of pea aphid management strategies, other than that of plant resistance. However, it is clear from the results of this study that there was a difference between the two geographical strains of *A. pisum* in their performance on the pea lines evaluated. These results also showed that certain resistance mechanisms, which were present in varying degrees in the various entries, influenced both the survival and growth of pea aphid. It was impressive to note the reaction of resistant lines 24 and 30. These two lines, that were resistant to the South African strain, were susceptible

to the Ethiopian strain. Furthermore, this also implies that the Ethiopian *A. pisum* strain should increase in population at a faster rate, thus killing susceptible genotypes more readily than the other strain. This may partially explain why the South African strain has not become a serious pest. The results also reinforce the necessity to take aphid genetic diversity into account in breeding programs for resistance in cultivated plants.

In small field plots, the major problem is that the uneven distribution of pea aphid infestation in space and time allows the possibility of chance escapes from pest damage to be incorrectly recorded as resistance. Genotypes showing low aphid counts or no damage in such tests therefore need closer scrutiny.

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Table 2.1 Varieties/lines and reference numbers for *Pisum sativum* screened for resistance against two geographical strains of *Acyrthosiphon pisum.*

Variety / line	Origin	Status	Reference number
Adi	Ethiopia	Released variety	1
Green Feast	South Africa	Released variety	2
G22763-2C	Ethiopia	Released variety	3
Hassabe	Ethiopia	Released variety	4
Holetta Local-90	Ethiopia	Released variety	5
Markos	Ethiopia	Released variety	6
Milky	Ethiopia	Released variety	7
Mohanderfer	Ethiopia	Released variety	8
NC 95 Haik	Ethiopia	Released variety	9
Oregon Sugar Pod II	South Africa	Released variety	10
Shield	South Africa	Released variety	11
Sugar Queen	South Africa	Released variety	12
EXDZ	Ethiopia	Breeding line	13
G22763X305ps210736-2	Ethiopia	Breeding line	14
HI-7	Ethiopia	Breeding line	15
HI-21	Ethiopia	Breeding line	16
JI-91	Ethiopia	Breeding line	17
JI-116	Ethiopia	Breeding line	18
JI-898	Ethiopia	Breeding line	19
KFP-103	Ethiopia	Breeding line	20
Kyondo	Ethiopia	Breeding line	21
NEP 874 UK	Ethiopia	Breeding line	22
Nur 74B x Filby	Ethiopia	Breeding line	23
304 WA 1101937	Ethiopia	Breeding line	24
305PS 210025	Ethiopia	Breeding line	25
305PS 210572	Ethiopia	Breeding line	26
305PS 210687	Ethiopia	Breeding line	27
305PS 210900	Ethiopia	Breeding line	28
061K-2P-2/9/2	Ethiopia	Breeding line	29
061K-2P-14/7/1	Ethiopia	Breeding line	30

Table 2.2 South African strain of *Acyrthosiphon pisum* population level scores and percentage of dead plants for 30 field pea genotypes in the greenhouse, scored 7, 14 and 21 days after infestation.

Entry	Entry code #	Aphid rating score (1-6) Days after infestation*				% dead seedlings
		7	14	21	Mean	
Adi	1	2.3	3.7	5.0	3.7	0
Green Feast	2	2.3	3.3	5.0	3.6	0
G22763-2C	3	2.7	4.0	5.0	3.9	0
Hassabe	4	2.3	2.7	4.3	3.1	0
Holetta Local-90	5	2.3	2.3	2.7	2.3	0
Markos	6	2.0	3.0	5.0	3.5	0
Milky	7	2.0	3.7	4.3	3.3	0
Mohanderfer	8	2.0	3.0	5.0	3.3	0
NC 95 Haik	9	3.0	3.7	5.3	4.0	0
Oregon Sugar Pod II	10	3.0	3.3	4.3	3.6	0
Shield	11	2.7	3.7	5.3	3.9	0
Sugar Queen	12	2.3	3.0	4.0	3.1	0
EXDZ	13	2.3	3.0	4.0	3.1	0
G22763X305ps210736-2	14	3.0	4.3	4.7	4.0	0
HI-7	15	2.3	3.3	4.3	3.3	0
HI-21	16	2.0	3.3	5.0	3.4	0
JI-91	17	2.7	3.7	5.3	3.9	0
JI-116	18	2.7	3.3	5.0	3.7	0
JI-898	19	2.3	2.7	3.0	2.7	0
KFP-103	20	3.0	3.3	4.0	3.4	0
Kyondo	21	2.0	3.0	4.3	3.1	0
NEP 874 UK	22	2.3	4.7	6.0	4.3	66
Nur 74B x Filby	23	2.3	3.7	4.0	3.3	0
304 WA 1101937	24	2.0	2.0	2.7	2.2	0
305PS 210025	25	2.7	4.0	5.7	4.1	33
305PS 210572	26	2.0	3.0	4.3	3.1	0
305PS 210687	27	2.0	2.7	3.7	2.8	0
305PS 210900	28	2.0	3.3	5.3	3.5	0
061K-2P-2/9/2	29	2.0	2.7	2.3	2.3	0
061K-2P-14/7/1	30	2.3	2.0	2.0	2.1	0
LSD (0.05)		0.84	1.34	1.53	1.00	
CV (%)		22.0	25.1	21.8	18.5	

* Score data are mean scores from 3 replicated blocks

Table 2.3 Ethiopian strain of *Acyrthosiphon pisum* population level scores and percentage of dead plants for 30 field pea genotypes in the greenhouse, scored 7, 14 and 21 days after infestation.

Entry	Entry code #	Aphid rating score (1-6) Days after infestation*				% dead seedlings
		7	14	21	Mean	
Adi	1	2.7	5.0	6.0	4.6	33
Green Feast	2	2.7	5.0	6.0	4.6	33
G22763-2C	3	3.3	5.0	6.0	4.8	33
Hassabe	4	3.0	4.3	6.0	4.4	66
Holetta Local-90	5	2.3	4.3	5.7	4.1	0
Markos	6	3.0	4.7	6.0	4.6	66
Milky	7	2.7	5.7	6.0	4.8	100
Mohanderfer	8	4.0	5.0	6.0	5.0	33
NC 95 Haik	9	3.0	4.3	6.0	4.4	66
Oregon Sugar Pod II	10	3.3	5.3	6.0	4.9	66
Shield	11	5.7	6.0	6.0	5.9	100
Sugar Queen	12	3.7	5.7	6.0	5.1	66
EXDZ	13	2.7	5.0	6.0	4.5	33
G22763X305ps210736-2	14	2.3	4.7	6.0	4.3	33
HI-7	15	3.0	5.3	5.3	4.6	33
HI-21	16	3.7	5.0	6.0	4.9	66
JI-91	17	3.0	4.3	6.0	4.4	66
JI-116	18	2.7	5.0	5.7	4.4	33
JI-898	19	3.3	4.7	6.0	4.7	66
KFP-103	20	2.3	5.0	6.0	4.5	33
Kyondo	21	4.0	5.7	6.0	5.2	66
NEP 874 UK	22	3.3	6.0	6.0	5.1	100
Nur 74B x Filby	23	3.7	5.7	6.0	5.1	33
304 WA 1101937	24	3.7	5.7	5.7	5.0	66
305PS 210025	25	3.7	6.0	6.0	5.2	100
305PS 210572	26	2.7	4.3	5.3	4.1	33
305PS 210687	27	2.7	4.7	5.3	4.2	0
305PS 210900	28	3.0	5.0	6.0	4.7	33
061K-2P-2/9/2	29	2.3	3.7	4.3	3.4	0
061K-2P-14/7/1	30	3.0	5.3	6.0	4.8	66
LSD (0.05)		1.32	1.55	0.55	0.86	
CV (%)		25.5	18.9	5.7	11.1	

* Score data are mean scores from 3 replicated blocks.

CHAPTER 3

Components and mechanisms of resistance in selected field pea, *Pisum sativum* L. lines to pea aphid, *Acyrthosiphon pisum* (Harris)

Abstract

Two strains of pea aphid, *Acyrthosiphon pisum* (Harris) from pea fields in South Africa and Ethiopia were tested to assess the mechanisms of resistance and genetic variation by measuring their responses to seven field pea lines. Seven life-history traits were assessed for each strain. Differences in response periods to these traits by the genotypes revealed that the two strains are genetically distinct. Nymphal development time, nymphipositional period and total life span were longer for the Ethiopian strain than for the South African strain. South African nymphs developed to adults between 8.4 and 9.8 days, whereas those in Ethiopia took 10.1 to 11.6 days (the ranges indicate variation in mean values among different lines). The adult nymphipositional period lasted 6.2 to 9.6 days in South Africa and 14.5 to 16.7 days in Ethiopia. Total life span was 18.4 to 21.4 days and 30.7 to 32.4 days for South African and Ethiopian strains, respectively.

Total fecundity was higher in the Ethiopian strain, 74.9 to 95.4 nymphs on lines 05 and 24, compared with the South African strain, 20.2 to 42.2 on lines 24 and the susceptible control respectively. Strain variation was also evident in tolerance and antixenosis resistance. The Ethiopian strain caused more stunting than the South African strain in all field pea lines. The South African strain was the least aggressive across all entries. The drastic difference in the Ethiopian strain's virulence on all test entries demonstrated how unpredictably resistant peas could perform at a particular geographic location.

Taking antibiosis, tolerance and antixenosis into account, the entries that performed best against *A. pisum* strains were lines 05 and 27. Strain variation was most apparent in the performance of line 24 and to a lesser degree to lines 29 and 19. These lines were resistant to the South African strain but susceptible to the Ethiopian strain. However, there were specific cases where tolerance, antibiosis or both appears to be active mechanisms of resistance.

Key words: *Acyrthosiphon pisum*, development, fecundity, strains, field pea, South Africa, Ethiopia.

Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris) is an insect pest of considerable economic importance in the production of field pea and lentil throughout many areas of Ethiopia. However, infestations are greatest in mid-altitude regions (1800-2200 m a.s.l.). Recommended methods for control of pea aphid include the application of insecticide and early planting (Ali & Habtewold 1994). Although insecticides are a reliable method for pest control, they are costly and have undesirable non-target effects. Moreover, in field pea growing regions of the country, most growers are subsistence farmers, and neither the crop nor the resources of the farmers can warrant the use of synthetic insecticides against aphids.

Russel & Morrison (1924) recognized host resistance as one of the most promising methods to control pea aphid in peas. Since this observation, numerous studies have been conducted to screen pea genotypes for resistance to pea aphid (Markkula & Roukka 1971, Bieri, Baumgartener, Bianchi, Delucchi & von Arx 1983, Dowens 1994). Some resistant pea germplasm sources have been evaluated to determine the categories of resistance in operation

(Dahms & Painter 1940, Campbell & Mackauer 1977, Leather & Dixon 1984, Soroka & Mackay 1991, Holtcamp & Clift 1993).

Of the thirty genotypes screened for resistances to the pea aphid (Chapter 2), seven showed various levels of resistance and the mechanisms governing the observed resistance were, therefore investigated in these lines including the susceptible check (Table 3.1). These seven lines were subjected to further tests for their toxic or otherwise detrimental effect upon the aphids (antibiosis), nonpreference (antixenosis) and their ability to withstand infestation, while still supporting insect populations that would severely damage susceptible plants (tolerance). Information about mechanism of resistance can be very important for decisions on how to handle resistant material in breeding and screening programs and may also affect decisions regarding deployment of resistance genes since there are different biotypes/clones of pea aphid capable of breaking resistance. The objective of this study was to elucidate the plant-related factors that determine the resistance or susceptibility to *A. pisum* in field pea lines identified as resistant in the preliminary stage of screening.

Materials and Methods

Antibiosis test. Colonies of the pea aphid, *Acyrthosiphon pisum* (Harris), were initiated from field-collected apterous viviparae from pea plants on Western Campus in the agronomy farm at the University of the Free State, Bloemfontein, South Africa in January 2000. The pea aphid colonies were cultured in the greenhouse on seedlings of Mohanderfer pea, a susceptible variety, at 20± 2^0C, 60-70% rh and a photoperiod of 16L:8D h. Tests for antibiosis, antixenosis and tolerance were conducted in the same environment as the one in which the screening study (Chapter 2) was conducted.

Three seeds of the seven lines were sown in plastic pots (15 cm diameter) filled with sterilized soil/peat mixture (1/1 volume/volume). Six days after sowing, seedlings were thinned to one per pot. Two weeks after sowing, each pot, representing one replication was enclosed with ventilated organdy bags (20 cm diameter by 80 cm height) made of a fine nylon mesh, and infested with three adults per seedling. The cages were fitted inside the plastic pots using iron stakes and tied with rubber band at the bottom to make them escape proof. Treatments (lines) were replicated 10 times and arranged in a randomized complete block design. All experiments were carried out in a greenhouse at the University of the Free State, between January and December 2000.

The aphids were allowed to larviposit for 24 h on test plants. After the appearance of offspring, all aphids except one first-instar nymph were removed. Singly caged, newborn aphids produced in this way were referred to as standard aphid. The individually caged entries containing these nymphs was placed in a greenhouse at $20^0C \pm 1^0C$, 50-60% rh and a photoperiod of 16L: 8D h. The nymphs were kept on the test plant until they had matured and were beginning to reproduce. For the purposes of this study, all nymphs that were produced within the 4-hour period, were assumed to be of uniform age.

The developmental rate of each nymphal instar was determined by checking for ecdysis every 24-h from birth until adulthood. From these data the mean duration of each of the nymphal instars was calculated. Daily reproduction was determined over the adult life span. Aphid survival was recorded daily, and the nymphs produced were counted and carefully removed without disturbing the adult. This was done every day until the female died. The prenymphipositional period of the adult was determined as the period between birth and the deposition of the first progeny. Plants were trimmed when they grew too large

for the cages so as to facilitate the removal and replacement of cages each time the aphid counts were made. In all experiments, when the upper 1-2 cm of the soil in the pots became dry, water was added carefully to the soil line so as not to wash aphids off the plants.

Parameters measured for making inferences about antibiosis included fecundity (mean number of nymphs produced per female), nymphipositional period (mean number of days from production of first progeny to last progeny), mean maximum number of nymphs produced in a single day, mean number of nymphs produced per day and longevity.

The intrinsic rate of increase (r_m) was estimated separately for each test entry, using the method of Wyatt & White (1977), i.e. $r_m = 0.74 (\log_e M_a/d)$, where d is the prenymphipositonal time, M_a is the number of progeny produced in time equal to the prenymphipositonal time, and 0.74 is a constant. Finite rates of increase (the number of individuals added to the population per female per day, or the population capacity to multiply a number of times per female per day) were estimated using the equation λ $\text{antilog}_e r_m$ (De Loach 1974). Generation time (Td) of *A. pisum* was computed using Td = 4d/3, where d is the prenymphipositonal time, whilst time for population to double its size (DT) was estimated using $DT = [\log_e (2)]/r_m$.

Statistical analysis was performed on each of the parameters measured using ANOVA (MSTAT-C, 1990) for making inferences about antibiosis, such as fecundity, nymphipositional period, mean maximum number of nymphs in a single day, mean number of nymphs produced per day and total life span. Means were compared using Duncan's Multiple Range Test (Duncan 1955) at a significant level of $P \leq 0.05$, unless otherwise stated.

In Ethiopia the culture of pea aphid that was used was collected from Holetta Agricultural Research Center, and maintained continuously on Mohanderfer field pea in the

same environment as the one in which the previous study was conducted. The same field pea lines (Table 3.1) were studied for their components of resistance to pea aphid in an insectary under natural photoperiods with mean temperature during the experiment of 22.7^{0}C (day) and 15.5^{0}C (night) and relative humidity of 70 – 94% from July to September 2000. Three seeds of each entry were planted in plastic pots (20-cm diameter) and upon emergence seedlings were thinned to the most vigorous one. The potting medium contained sterilized soil with 250 mg Diammonium phosphate (18 N, 46 P_2O_5) per kg of soil added (T. Bekele, Holetta Agricultural Research Center, personal communication). The pots were then caged with clear plastic cylindrical cages (15-cm diameter and 60 cm high). The screen cages had holes covered with a fine mesh screen on the top and six sides for ventilation. Plants were watered when the soil was dry to the touch. The other procedures followed were as above.

The role of non-preference, tolerance and antibiosis in field pea resistance to pea aphid was examined in the insectary, using the seven selected cultivars. Studies on antibiosis involved rearing cohorts of apterae aphid on different cultivars and comparing them in respect of the number of aphids surviving to reproduce, the fecundity, the duration of nymphiposition, the net reproductive rate, the intrinsic rate of increase, the finite rate of increase and doubling time.

Antixenosis Test. *Acyrthosiphon pisum* used in these tests were adult, apterous viviparae from the same colony as for the antibiosis experiment. Schweissing & Wilde (1979) stated that antixenosis tests with apterous *Scizaphis graminum* (Rondani) closely approximated the results obtained with alates. Apterous *A. pisum* are, moreover, routinely used in antixenosis tests.

This study consisted of a free-choice experiment to examine antixenosis properties of the varieties. Single pea seedlings of seven field pea lines were planted in equidistant hills in a circular pattern around the edge of plastic pots (26 cm in diameter), for a total of seven entries per pot. The arrangement of the seedlings was randomized independently in each pot. The experimental design was a randomized complete block (RCB) with 10 replications. When plants were 15 days old, aphids were released in the center of each pot so that all entries had an equal opportunity of being infested by the aphids. Plants (15 days old) in ten pots were infested by placing 70 *A. pisum* late last instar in the center of the circle in each pot resulting in a mean of 10 aphids per plant. The pots were covered with organdy bag and cylindrical cages as described previously. The number of aphids per plant was recorded after 24, 48, and 72 hours. First instar nymphs were not counted as indicators of host preference. The experiment began after sunset to avoid possible aphid phototaxis.

Data were analyzed using the analysis of variance (MSTAT-C) package. Data was transformed to square root (n+0.5) to stabilize the variance when appropriate. When F values were significant ($P< 0.05$), means were compared using Duncan's Multiple Range Test (DMRT).

Tolerance Test. In the tolerance experiment, each of the seven entries was planted in plastic pots (15-cm diameter). The experimental design was a randomized complete block with 5 replications. At two weeks after planting (plants 9-10 cm tall), one of two plants of similar height per entry was caged with 10 live adult apterous pea aphid and the other plant was caged without pea aphid (control). Because of variability in plant height, control plants were paired as closely as possible so each replication of infested and control plants from the

same test entry was approximately the same height. The pots were covered as described in the antibiosis test above.

Plants were examined every 48 h to maintain 10 aphids in the cages on the infested plants and to remove any excess aphids from plants. Uninfested plants were treated in the same manner as infested plants (i.e.cages were removed every 48 h and replaced to mimic the treatment of infested plants). Three weeks later, plant height, fresh plant mass, dry plant mass, number of leaves, leaf area and root biomass were recorded for infested and non-infested plants. After measuring fresh and root mass, plants were dried in an oven at 50^0C for 72 h and weighed. All the measurements were standardized by dividing uninfested plant values by infested values [e.g. percentage of the height in uninfested plants = (1-[uninfested-infested/uninfested]) x 100). To measure leaf area, individual leaves were cut from the plants and placed flat in a clear transparent conveyor belt of a Li-Cor Model 3100 Area Meter (Li-Cor, Inc., Lincoln, Nebraska). The experiment was designed as a one factor randomized complete block with five replications. Data were analyzed as described in the antixenosis test.

Results

Antibiosis. In the South African pea aphid strain contrasts between the susceptible line and the selected resistant lines indicated that there were significant differences between susceptible and all resistant lines examined for all parameter comparisons (except longevity). Differences among lines relative to number of days before female began producing nymphs were significant ($P < 0.05$). Aphids developed significantly faster on the susceptible line (8.4±0.5 days) and slower on line 27 (9.8± 1.2 days) and the remaining were intermediate

(Table 3.2). On all lines tested pea aphids reached maturity between eight and nine days after hatching.

The duration of the mean nymphipositional period and the mean number of days in which nymphs were produced were also significantly different among the lines evaluated. Aphids fed on line 05 produced nymphs for a significantly longer period (9.6± 2.1 days) than all the remaining entries, excluding the susceptible check (line 22). The remaining entries were not significantly different from one another. However, aphids on line 27 and 29 reproduced relatively quickly and died soon after producing their last offspring. The nymphipositional time was comparable on line 27 and 29 (6.4 days on line 27 vs 6.2 on line 29), but was significantly different from the susceptible and 05 lines.

The most apparent differences were found in the total number of progenies produced per female. Differences among lines for aphid fecundity were highly significant ($P<0.001$). Fecundity was reduced in adults reared on the resistant lines, indicating appreciable levels of antibiosis in these genotypes. In this test the mean ± SEM fecundity over the lifespan of an adult pea aphid ranged from 20.2± 3.5 nymphs on line 24 to 42.2± 5.2 on the susceptible check with an average of 27.0± 1.8 nymphs across all test entries (Table 3.2). The mean number of progeny produced per aphid averaged across all six entries (24.5) was significantly less than the mean progeny number on the susceptible line (42.2). Significantly fewer nymphs were produced on lines 27, 29, 30 and 24 than line 05. These resistant lines (27, 29, 30 and 24) showed the highest level of antibiosis and the number of progeny per aphid on these entries differed significantly from the progeny number on line 05. Among the field pea lines tested, line 29 and 24 had the highest level of antibiosis followed by lines 27, 30 and 19, which were similar in their level of antibiosis to the pea aphid.

Significant differences were observed in the maximum number of nymphs produced in a 24-h period by a single female among plant lines (Table 3.2). Nymphs were significantly less ($P<0.05$) on resistant lines (range 5.4 to 8.4) than on the susceptible check (11.2). The maximum daily nymphal production on line 24 (5.4) was significantly less than on lines 27 (8.2), 30 (8.4), and 19 (7.8). When assessed on a per-day basis, mean daily nymphal production per day was significantly lower on lines 27, 29, 30, 19 and 24, than on the susceptible line. Aphids fed on line 05 were intermediate in nymphal production (i.e. 3.7 nymphs/day). The apterous adults attained the greatest mean progeny production per day of 5 females per female on the susceptible check line. When the resistance of the five lines (27, 29, 30, 19, and 24) was assessed using the rate of increase per female per day, they did not differ significantly in their resistance. The susceptible check (line 22) and line 05 were also different from each other (5.0 and 3.7 female/day respectively). Survival of *A. pisum* was similar among the lines evaluated (range 18.4 to 21.4 days with a mean of 20.1 days) (Table 3.2). The longevity of the reproducing adult was not significantly associated with cultivars, but survival on line 29 was shorter than those on the other lines tested.

Values of the intrinsic rate of increase (r_m) listed in Table 3.3 were computed on a daily basis. The figures were converted to finite rates of increase (λ), i.e. the number of individuals added to the population per female per day, based on the equation $\lambda = \text{antilog}_e\ r_m$. For example, for the susceptible check, λ = antilog 0.330 = 1.39, or the population has the capacity to multiply 1.39 times per female per day. At the end of one week each female could contribute 1.39^7, or 19.4 individuals to the population.

The r_m of *A. pisum* was significantly ($P<0.05$) lower on the resistant lines than the susceptible line and line 05 (Table 3.3). Differences in r_m values on different lines were

caused primarily by differences in patterns of fecundity. The antibiosis components of lines 29 and 24 seemed to be slightly greater than that of lines 27, 30 and 19, although these differences were not significant. The r_m was significantly lower for pea aphid populations reared on lines 27 and 24 than for those reared on the susceptible check line and line 05. Small differences in the r_m will result in large changes in the size of populations. In this experiment, aphid population size would be greatly reduced on resistant lines compared with the susceptible check line because of the 18-26% reduction in r_m values, with the exception of line 05. The other demographic statistics did not show appreciable differences among the entries evaluated (Table 3.3).

The difference in fecundity is too large to consider these lines as resistant genotypes in our study. Such differences, for example, could potentially lead to the number of aphids on the susceptible line being twice as high as that on the resistant lines. All that these results showed is that in our experimental conditions, these lines are less suitable for development and reproduction of the South African strain of pea aphid, as compared to the susceptible check and line 05.

A detrimental effect on the biology of the pea aphid indicates antibiosis. Antibiosis in this study was expressed as (1) an increase length of time to reach reproductive maturity (2) decreased fecundity (3) rate of increase and (4) combination of life table.

Nymph production of the Ethiopian pea aphid strain on field pea lines is summarized in Table 3.4. Total nymph production over the entire aphid life span to be a measure of antibiosis. The results indicated that lines 29 and 24 had significant lower antibiosis than lines 05, 27 and 30. However, nymph production on lines 22 and 19 were not significantly different from that on lines 29 and 24. There was a significant difference ($P<0.05$) in

antibiosis as measured by the number of nymphs produced per adult, which ranged from an average of 95.4±9.1 nymphs from adults fed on line 24 to 74.9±9.0 nymphs from adults fed on line 05, with an overall mean of 84.7±7.5. In this experiment, the Ethiopian strain produced a maximum of 95.4 and 93.6 nymphs on line 24 and 29 respectively, while the least was on line 05 (74.9). There were no significant differences in the number of nymphs produced per female per day, number of days in nymphipositional period and longevity. No comparison of means was carried out as treatments did not differ significantly.

The r_m (intrinsic rate of increase estimates) of *A. pisum* on all the lines was not significant, indicating that separation of lines is not possible having the various combinations of antibiosis. The r_m value of the susceptible line was the lowest (0.284), although these differences were not significant with the remaining lines. Mean generation and doubling times were similar among aphids reared on the seven genotypes. Mean generation time was the lowest in aphids feeding on line 24, when compared to those reared on other lines (Table 3.5). The mean doubling times were also similar on the lines, with average among lines being < 1 day.

Lifetime fecundity was higher in the Ethiopian strain than in the South African strain (Figure 3.1). The South African strain produced 42.2 and 24.8 nymphs on the susceptible and resistant (line 27) lines respectively, whereas the Ethiopian strain produced 86.9 and 77.4 nymphs on these same lines. The mean daily nymphal production on the seven lines was lower ranging from 2.2 to 5.0 for the South African compared with Ethiopian strain (range 5.2 to 6.2). The peak daily fecundity on the susceptible line was similar for the two strains. In general, the tested pea aphid strains performed differently on field pea entries with regard to most life-history parameters (Table 3.4 and 3.5).

Antixenosis. Table 3.6 contains data for antixenosis in the South African pea aphid strain on field pea lines. Not all the introduced *A. pisum* were recovered at the end of the test, but 614 (88%) were recovered from 700 individuals. The analysis of variance of this experiment showed no significant difference between the numbers of *A. pisum* on any of the test entries 24 h after infestation. However, at 48 and 72 h the differences were significant ($P<0.05$). The highest number of aphids was recorded on the susceptible line (11.6) followed by line 30 (9.4). As a group, the resistant lines were not significantly different from one another.

The numbers of adults per plant at 72 h after release ranged from 6.6 ± 2.4 on line 29 to 11.6 ± 2.9 on the susceptible line and were significantly different ($P<0.05$) (Table 3.6). Apparently the aphids selected nearby plants soon after they were released and had not moved enough to show a behaviorally based preference at 24 h. After 48 h, some aphids had left the plants, resulting in a decrease in aphids per plant on all entries. The decrease in aphids on some of the lines (for example line 29 and 30) was substantial. All test entries (except lines 29 and 30) had significantly fewer adults than the susceptible check line. The susceptible line was thus preferred for settling and reproduction. The two sources with the highest level of antixenosis (least preferred) were lines 27 and 29 (Table 3.6). This clearly shows the involvement of antixenosis (non-preference) as a mechanism of resistance to pea aphid in field pea.

Correlation among evaluation times was highly significant. Correlations between 24- and 48-h antixenosis ratings were similar to correlation between 24- and 72-h ratings ($r = 0.59$ and 0.60, $P < 0.001$), respectively, while correlations between 48- and 72-h ratings were relatively larger ($r = 0.85$, $P < 0.001$).

In the Ethiopian strain the antixenosis test (Table 3.7) indicated that pea aphids required only 24 h to select a preferred line. There were significant differences among the seven entries for antixenosis. Here also not all released *A. pisum* adults were recovered at the end of the test, but 601 (85.9 %) were recorded from 700 individuals. These figures are very close between the two strains. Aphids showed preference soon after they were released at 24 h. Twenty four hours after release, line 24 had a significantly higher number of aphid (10.2±1.9) when compared to the remaining lines, except line 30. The numbers of adults per plant 48 h after release ranged from 8.4±2.5 on line 24 to 5.1±1.0 on line 05 and were significantly different. After 72 h, very few aphids had left the plants, resulting in a negligible decrease in aphids per plant on all entries. Line 24 consistently sustained the highest number of aphids for settling and development. This shows the involvement of antixenosis (non-preference) as a mechanism of resistance to the pest in field pea. Correlation among evaluation times was significant. Correlations between 24- and 48-h ratings for antixenosis were different to correlation between 24- and 72-h ratings ($r = 0.41$, $P < 0.01$; $r = 0.28$, $P < 0.05$), respectively. Correlation ratings between 48- and 72-h were larger and highly significant ($r = 0.62$, $P < 0.001$).

Tolerance. At the beginning of the experiment for the South African pea aphid strain plants of all seven tests entries were at growth stage 102 (Knott 1987). When the test was stopped the plants were at growth stage 112. Prior to infestation, at the onset of the tolerance test, there was no significant difference ($P<0.05$) in plant height between plants of the same lines (Table 3.8). Although plant root mass and leaf area were measured, plant height, leaf number and plant mass were the most consistent between replications and considered the most suitable measurement in assessing tolerance in the field pea lines.

The entries showed significant differences for tolerance at the seedling stage 21 days after infestation (Tables 3.9 & 3.10). In general, plant growth was retarded in infested plants (56.9 cm in height) compared with uninfested control (79.2 cm in height). Growth of infested entries ranged from 52.6 cm with line 22 (susceptible) to 63.0 cm with line 24 with a test average of 56.9 cm (Table 3.9). Plant growth was significantly retarded in infested plants of line 29 compared with lines 27, 22, and 24. Mean plant height, recorded 21 days after infestation for the susceptible line was 76.9% of the height of the control, and mean plant heights of line 27 was 81.9% of that for the control plants. Although lines 29 and 30 had the greatest antibiosis effect they had the highest percentage of height reduction. The susceptible line 22 was more tolerant, but had the highest number of nymphs produced in the antibiosis test and also had more aphids per plant in the antixenosis test than was anticipated. However, in the preliminary screening, *A. pisum* severely damaged and even killed some plants of this line in the greenhouse.

Similarly, the mean number of leaves was significantly affected by *A. pisum* feeding. Leaf number of infested entries ranged from 42.4 with line 19 to 52.0 in the susceptible line with an overall mean of 46.1. Plants of lines 30 and 19 suffered the highest reduction and were almost twice that of lines 05, 22 and 24. The three lines 05, 22 and 24 were least affected and significantly lower than lines 29, 30 and 19.

Mean dry plant weight for control plants and infested plants were 1.249 and 1.105 g, respectively. Except for line 29, the remaining lines showed higher weight than uninfested control plants, although data for line 27 suggested that the aphid may promote dry plant weight. (Table 3.10). Root fresh and dry mass and leaf area were not consistently affected by

the presence of *A. pisum* at these densities and duration of infestation, although for some lines suggested that the aphid may also promote root growth.

In the Ethiopian strain there was no significant variation in plant height at the beginning of the experiment and therefore the differences in plant height in the first 21 days was caused by the effect that aphid feeding had on the field pea lines (Table 3.12). Significant differences were noted between uninfested tolerance test entries in plant growth, number of leaves, fresh and dry plant mass at the end of the test, indicating that the genotypes are not similar in these parameters. Stunting was very severe among the field pea lines, and some resistant lines showed more stunting or equal stunting to that of the susceptible line. The average plant growth of all uninfested test entries was 68.0 cm, whilst for the corresponding infested plants it was 21.4 cm. Growth of infested entries ranged from 16.1 cm in line 22 to 27.6 cm in line 29, with a test average of 21.4 cm (Table 3.12). In the uninfested plants, growth of line 22 was significantly lower than that of line 19. In the infested plants, lines 29, 30 and 19 had significantly more growth than the remaining lines. With the exception of line 22 which was at 74%, the growth of all the other infested field pea lines was at least 69 % that of the uninfested plants. Percent plant height confirmed that pea aphids caused significant reduction in plant height in the Ethiopian strain, when compared to the South African strain (Figure 3.2).

Significant genotype differences ($P<0.05$) were observed in the number of leaves between infested and uninfested field pea lines. Similarly, the leaf number of infested entries ranged from 14.0 in line 27 to 30.6 in line 29, with an overall test average of 22.7. Number of leaves was significantly ($P<0.05$) lower in infested plants of lines 05 and 27, compared with lines 29, 30, 19 and 24.

Significant genotype differences were also evident in plant weight data. Fresh plant weight was significantly higher for line 29 than in all entries with the exception of line 30 (Table 3.13). Mean fresh weights for the control and infested plants were 3.05 and 0.92 g, respectively. The fresh plant mass of infested entries ranged from 0.43 g in line 05 to 1.55 g, in line 29, with a mean of 0.92 g. With the exception of lines 27 and 30, which respectively showed 35.6 % and 38.6 % dry weight of uninfested plants, line 29 showed significantly more dry weight (58.8 %) than all the remaining lines.

Resistance Index. Table 14 shows normalized indices for antibiosis, tolerance, and antixenosis for South African pea aphid populations. The resistance index (RI) ranks the lines in terms of the combination of their resistance components and does not indicate any statistical differences. Antibiosis, antixenosis, and tolerance were determined for 7 lines of test entries. However, because the three resistance components were measured in different scales (nymphs/female, aphids/plant and plant damage), the data for each component were first normalized to a common scale by dividing each value from a series of test entries by the highest value occurring for that resistance mechanism. The resulting values were designated as mechanism indices. A plant resistance index was then calculated for each entry using the following equation: 1/(xyz), where x is the antibiosis index, y is the antixenosis and z is tolerance indices (Inayatullah, Webster & Fargo 1990).

The use of the plant resistance index in Table 3.15 is a simplified way to simultaneously present and evaluate all three resistance mechanisms, and provides a single value to aid in determining appropriate resistant lines for crosses (Inayatullah *et al.* 1990). In this case, all mechanisms were weighted equally, but if a particular component was to be deemed more important in a breeding program, it could be weighted accordingly. The

resistance indices in Table 3.15 show the superior resistance level of the resistant lines (except line 05), compared with the susceptible line in the test. Line 05 had a similar resistance index to that of the susceptible line and was thus considered unsuitable as a resistance source. In general, the five lines (27, 29, 30, 19, and 27) appeared to have the greatest levels of pea aphid resistance when taking all resistance mechanisms into consideration.

For the Ethiopian population of pea aphid, Table 3.16 shows the normalized indices for the three components of resistance. Based on its tolerance components, the data indicate that lines 05, 27 and 22 are more resistant than the remaining lines. The most susceptible lines appeared to be 29, 16, 30, 19 and 24, albeit that these lines were not as resistant in these tests as they were in the field. In part, this lack of resistance appears to have been the result of their low antibiosis.

Discussion

Survival of *A. pisum* depends on the suitability of its host for feeding and reproduction. Several authors have reported differences in host suitability for survival and reproduction. For example, Soroka & Mackay (1991) examined six cultivars of peas regarding suitability for one strain of pea aphid. All measurements of pea aphid performance were affected by pea varieties. Similar results have been reported by Damte (1999) and Zeng, Pederson, Davis & Ellsbury (1993) who studied differences in suitability for pea aphid survival and reproduction between lentil and red clover varieties. The results from this study agree with those presented by others and strongly suggest the existence of a genetic component that determines the suitability of the host plant for pea aphid survival and reproduction.

Reproductive rate of pea aphid for the South African strain was significantly lower on the resistant line compared with the susceptible control (line 22). The differential rate partially explains the antibiosis mechanism of resistance that was observed in lines 27, 29, 30, 19 and 24. The susceptible line was the best host for the pest, as illustrated by the shortest development time and highest mean fecundity for this strain. In this study, fecundity rates and total mean fecundity of pea aphid were highest on the susceptible line and line 05. The lower fecundity rate on resistant lines suggests that these lines may contain a toxin, or toxin may be induced in the plants due to aphid feeding.

Line 29 had the greatest *A. pisum* resistance of the lines evaluated and showed antibiosis, tolerance and antixenosis. It was noted that aphids were more restless and spent less time feeding on this line than on any other lines. They were also dislodged from the plants more easily and more were lost or escaped from the pots. All six resistant lines show a high level of antibiosis (reduced daily nymphal production) even though the aphids had a slightly longer developmental period than the susceptible control. Similarly, pea aphids reared on resistant peas exhibited lower fecundity (ξ 38 nymphs/adult) than when reared on susceptible peas (ξ 55 nymphs/adult) (Soroka & Mackay 1991). Zeng *et al.* (1993) also found lower fecundity of pea aphids on resistant than on susceptible red clover (range 20-27). In this study relatively lower number of nymphs per adult were obtained than was reported on peas by Soroka & Mackay (1991) and on alfalfa by Girousse & Bournoville (1994), possibly because of differences in pea aphid clones/strains and species of host plants used to culture the aphid.

The screening tests revealed only small differences in host suitability among seven pea lines for the Ethiopian strain. Variation in aphid fecundity was the clearest expression of

antibiosis resistance among the parameters tested for this strain. This is probably the single most important factor in the fluctuating intrinsic rate of increase. Based on this parameter, lines 05, 27 and 30 are more resistant or less suitable for pea aphid survival than the remaining lines. The susceptible line was also the best host for the pea aphid as demonstrated by the highest mean fecundity. The mean values of progeny per aphid nymphal production (range 77-97) reported by Girousse & Bournoville (1994) on two cultivars of alfalfa and Newman & Pimentel (1971) on peas (range 78-113) were close to the values in this study.

Soroka & Mackay (1991) and Sandström (1994) reported a pea aphid nymphal development time of 8.4 to 11.0 days and 7.8 to 8.7 days respectively, which are similar to the values found for pea aphid on peas in this study. Furthermore, these authors reported a nymphipositional period of 11.3 to 13.8 days, which is longer than that of the South African strain (6.2 to 9.6 days), but shorter than that of the Ethiopian strain (14.5 to 16.7 days). Even differences in nymphipositional time as small as 24 h can have a considerable effect on population growth, as shown in a simulation by Wiktelius & Pattersson (1985) for the aphid *Rhopalosiphum padi*. These differences might be caused by different experimental conditions, pea aphid populations or host plant influences on pea aphid (or all three). Newman & Pimentel (1971) reported a longer pea aphid longevity (27 to 34 days) than was found for the South African and Ethiopian strains (18 to 21 days and 28 to 32 days respectively). However, Soroka & Mackay (1991) report a total life span of 20 to 26 days, a value closer to that of this study.

The calculated natural intrinsic rate of increase (r_m) followed the rankings of the lines for each parameter. The r_m values obtained for the South African strain are relatively lower

(except in the susceptible line), when compared with that of Ethiopian stain. The r_m values presented in this study are within the range of r_m estimates presented for different pea cultivars under similar temperatures (Soroka & Mackay 1991, Morgan, Walters & Aegerter 2001). However, Sandström (1994) reported higher estimates of 0.322 to 0.372, whilst Hutchison & Hogg (1984) reported a r_m value as high as 0.380 on alfalfa. The results of the present studies are regarded as incomparable, since these authors used different host plants.

The antixenosis detected on field pea line 29 also occur in the field and could influence the initial infestation level of pea aphids. However, the antixonesis detected for this line is unlikely to make a significant contribution to field resistance, since *A. pisum* can be reared successfully on this line. Given a choice of host, *A. pisum* might select a genotype other than line 29, but when given no choice, it is able to survive and reproduce on this line.

All lines were less tolerant to pea aphid feeding as indicated by plant height and percentage of weight reduction. Based on plant height reduction, the selected lines ranged from tolerant to the South African strain to moderately tolerant to the Ethiopian strain. All lines showed more or less similar stunting, except for line 29 that was not severely stunted by pea aphid damage. Uninfested plants of most lines grew more than twice as tall as infested plants. Lines 29, 30 and 19 also showed low to moderate levels of tolerance based on comparison to percentage of uninfested plant height after pea aphid feeding.

In this study, few field pea lines have been found that display the three categories of resistance. Soroka & Mackay (1991) showed both antibiosis and antixenosis, but tolerance was not found in any of the lines they evaluated. Holtcamp & Clift (1993) reported that antibiosis and antixenosis are the main mechanisms of resistance of alfalfa cultivars to pea aphid, whilst Maxwell, Jenkins & Parrott (1972) reported similar results, but found that

tolerance is involved to a much lesser degree. Zeng *et al.* (1993) reported that one red clover line (N-2) showed antibiosis resistance. However, no tolerance and antixenosis assessments were conducted on this line.

Tolerance as a mechanism of resistance, may also provide resistance that is more stable than antibiosis or antixenosis (Smith 1989). Combining multiple categories of resistance in a single cultivar may prolong the resistance to *A. pisum* in adapted cultivars. The lack of high levels of reproductive antibiosis should negate or delay the development of *A. pisum* biotypes, and the tolerance response of these resistant sources should enable the aphids to survive on plants that will support predators and parasitoids populations. Although these reductions may not be great, they may be important under light to moderate field infestation levels when they are combined with the effects of pea aphid biological control agents (van Emden 1990).

Host-plant resistance at the levels discussed in this study may become an important component of an IPM system because of its compatibility with the use of natural enemies (Dodd & van Emden 1979, van Emden 1990, Messina & Sorenson 2001). Methods based on partial host-plant resistance may help limit aphid populations to acceptable levels on pea crop, as well as imposing less selection pressure for the development of resistant aphid biotypes (Lammerink 1968, Dunn & Kempton 1972). Results suggest that antibiosis in field pea lines are affected by decreasing population rate. Further examination of the mechanisms of antibiosis (e.g. toxins, growth inhibitors, reduced nutrient levels, hypersensitive plant growth responses, or plant structure factors) is needed to assess the effect of antibiotic field pea resistance in *A. pisum* population development.

In conclusion, the original greenhouse selections (Chapter 2) based on screening for resistance in the South African pea aphid strain, have been verified in the mechanism of resistance experiments. Line 29 had a high level of antibiosis, antixenosis and tolerance, whilst line 22 was susceptible to pea aphid in terms of the three mechanisms of resistance. The former line may be a useful source of resistance to pea aphid, and lines 27, 19 and 24 showed intermediate levels of resistance to pea aphid when the three components are considered. Taking antibiosis, tolerance and antixenosis into account, lines 27 and 05 were the most resistant lines for the Ethiopian strain.

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Table 3.1 Lines and reference numbers for *Pisum sativum* studied for mechanisms of resistance to South African and Ethiopian strains of *Acyrthosiphon pisum.*

Test Entry	Origin	Reference number
Holetta Local-90	Ethiopia	05
305PS210687	Ethiopia	27
061K-2P-2/9/2	Ethiopia	29
061K-2P-14/7/1	Ethiopia	30
JI-898	Ethiopia	19
304WA1101937	Ethiopia	24
NEP874UK (susceptible check)	Ethiopia	22

Table 3.2 Mean ± SEM life table characteristics of pea aphid confined on 7 field pea lines in the greenhouse, Bloemfontein, South Africa.

Entry Code #	Fecundity*	DNP	NP	Longevity	MDNP	PNP
05	31.6±5.1**b**	3.7±0.8**b**	9.6±2.1**a**	21.4±3.0	6.0±0.9**bc**	8.6±0.8**ab**
27	24.8±5.8**c**	2.6±0.8**c**	6.4±0.8**b**	19.8±1.1	8.2±2.2**b**	9.8±1.2**a**
29	21.8±6.5**c**	2.5±0.6**c**	6.2±2.0**b**	18.4±2.7	6.4±1.3**bc**	8.8±0.7**ab**
22	42.2±4.7**a**	5.0±0.7**a**	8.0±0.7**ab**	21.0±1.1	11.2±1.3**a**	8.4±0.5**b**
30	24.0±4.8**c**	2.8±0.7**c**	7.2±1.3**b**	19.8±3.0	8.4±1.8**b**	8.8±1.2**ab**
19	24.6±2.4**c**	2.7±0.5**c**	6.8±1.4**b**	20.6±1.5	7.8±1.0**b**	9.4±1.0**ab**
24	20.2±3.5**c**	2.2±0.5**c**	7.0±0.6**b**	21.1±1.5	5.4±1.3**c**	9.2±0.7**ab**
Mean	27.0±2.2	3.1±0.3	7.2±0.7	20.3±1.1	7.6±0.7	9.0±0.4
CV (%)	18.1	20.7	22.5	12.0	22.2	11.3

*Means in each column followed by the same letter are not significantly different (P = 0.05). Fecundity is the number of nymphs produced per female; DNP is the number of nymphs produced per female per day; NP is the number of days in nymphipositional period; Longevity is the number of days the adult aphid lived; MDNP is the maximum number of nymphs produced per female during a 24-h period; PNP is the number of days before female began producing nymphs. Coefficient of variation (CV).

Table 3.3 Demographic statistics derived from the life table study of individual pea aphids confined on seven lines of field pea, Bloemfontein, South Africa.

Entry Code #	r_m[a]	λ[b]	T[c]	DT[d]
05	0.297ab*	1.35	11.5	2.3
27	0.246c	1.27	13.1	2.8
29	0.259bc	1.30	11.7	2.7
22	0.330a	1.39	11.2	2.1
30	0.270bc	1.30	11.7	2.6
19	0.269bc	1.29	12.3	2.6
24	0.243c	1.27	12.3	2.8
Mean	0.273			
CV (%)	13.18			

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

[a] Intrinsic rate of increase

[b] Rate of increase per female per day (finite rate of increase)

[c] Mean generation time, days

[d] Doubling time

Table 3.4 Mean fecundity, daily nymphal production, nymphipositional period, longevity, maximum daily nymphal production and pre-nymphipositional period for *Acyrthosiphon pisum* on seven lines of field pea from Holetta, Ethiopia.

Entry Code #	Fecundity	DNP	NP	Longevity	MDNP	PNP
05	74.9±9.0**d***	5.2±1.1	15.0±3.1	30.9±2.6	10.9±1.9	10.6±0.7
27	77.4±14.9**bcd**	5.4±1.0	14.5±1.8	31.0±1.2	10.0±1.7	10.7±0.4
29	93.6±9.2**a**	5.5±0.7	16.7±1.9	32.0±3.0	11.6±2.3	10.5±0.9
22	86.7±11.7**abcd**	5.5±0.7	15.6±1.2	31.7±2.4	10.7±2.1	11.1±0.6
30	76.6±12.0**cd**	5.1±0.8	15.9±1.8	32.4±2.0	10.4±1.5	10.9±0.8
19	88.1±8.7**abc**	5.8±0.6	15.2±1.9	32.0±2.5	11.2±1.0	10.5±0.7
24	95.4±9.1**a**	6.2±0.7	15.6±2.1	30.7±2.0	11.4±1.0	10.1±0.3
Mean	84.7±7.5	5.5±0.3	15.5±0.6	31.5±2.1	10.9±0.5	10.6±0.3
CV (%)	13.6	15.0	14.3	8.2	16.4	6.5

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)
Fecundity is the number of nymphs produced per female; DNP, number of nymphs produced per female per day; NP, number of days in nymphipositional period; Longevity, number of days adult aphid lived; MDNP, maximum number of nymphs produced per female during a 24-h period; PNP, number of days before female began producing nymphs.

Table 3.5 Demographic statistics derived from the life table study of individual pea aphids confined on seven lines of field pea from Holetta, Ethiopia in 2000.

Entry Code #	r_m[a]	λ[b]	T[c]	DT[d]
05	0.292	1.24	14.3	2.4
27	0.291	1.34	14.3	2.4
29	0.304	1.35	14.0	2.3
22	0.284	1.33	14.8	2.4
30	0.293	1.32	14.5	2.5
19	0.305	1.35	14.0	2.3
24	0.318	1.38	13.5	2.2
Mean	0.300			
CV (%)	8.21			

[a] Intrinsic rate of increase

[b] Rate of increase per female per day (finite rate of increase)

[c] Mean generation time, days

[d] Doubling time

Table 3.6 The mean number ± SEM of *Acyrthosiphon pisum* per plant at 24, 48 and 72 h after infestation from Bloemfontein, South Africa.

Entry Code #	Number of *A. pisum*		
	24 h	48 h	72 h
05	10.8	9.4±3.8ab*	8.9±4.2ab
27	6.0	6.7±2.9b	6.7±2.2b
29	8.2	7.8±3.1b	6.6±2.4b
22	9.7	12.5±4.3a	11.6±2.9a
30	11.5	9.6±1.7ab	9.4±1.2ab
19	6.5	8.7±2.9b	7.7±3.4b
24	8.8	8.4±3.7b	8.4±3.4b
CV (%)	51.8	41.3	38.7

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.7 The mean number ± SEM of *Acyrthosiphon pisum* per plant at 24, 48 and 72 h after infestation from Holetta, Ethiopia.

Entry Code #	Number of *A. pisum*		
	24 h	48 h	72h
05	4.8±1.5c*	5.1±1.0b	4.9±1.8cd
27	7.4±2.4bc	6.5±2.2ab	6.2±1.4bcd
29	6.2±2.7bc	5.4±2.2b	4.3±1.3d
22	6.9±2.0b	6.9±1.8ab	7.5±1.3ab
30	8.3±2.0ab	7.2±1.5ab	7.3±1.5ab
19	7.7±1.4b	6.5±2.1ab	8.3±2.2a
24	10.2±1.9a	8.4±2.5a	8.4±2.1a
Mean	7.4±0.75	6.6±0.69	6.7±0.63
CV (%)	30.33	31.25	28.39

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.8 Plant height (cm) of field pea lines at the onset of the tolerance trial (Bloemfontein, South Africa).

Test entry	Plant height (cm)	
	To be infested	Not to be infested
05	10.8	9.4
27	10.0	10.4
29	11.2	8.1
22	9.6	9.9
30	8.1	8.7
19	9.8	8.7
24	9.6	9.5
Mean	9.9±0.92	9.2±0.73
CV (%)	16.6	19.5

Table 3.9 Tolerance component of resistance of field pea entries to pea aphid at Bloemfontein, South Africa in 2000.

Entry Code #	Plant Growth, cm		% Uninfested plants
	Uninfested	Infested	
05	77.4± 11.8abc*	53.6± 8.1	69.2ab
27	70.6± 8.9bc	57.8± 8.4	81.9c
29	88.05± 5.7a	55.6± 9.4	63.2a
22	68.4± 7.0c	52.6± 6.3	76.9bc
30	86.2± 3.9a	59.8± 10.3	69.4ab
19	80.2± 9.2ab	56.4± 10.8	70.3ab
24	84.0± 3.0a	63.0± 10.8	75.0bc
Mean	79.2± 3.6	56.9± 4.7	72.3
CV(%)	10.3	18.6	22.6

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.10 Tolerance component of resistance of field pea entries to pea aphid at Bloemfontein, South Africa in 2000.

Entry Code #	No. leaf infested	% Uninfested	Fresh plant weight (g) infested	% Uninfested	Dry plant weight (g) infested	% Uninfested
05	45.8± 5.1ab*	89.4bc	6.5	96	1.03	102
27	44.4± 4.5b	84.8ab	7.5	117	1.20	121
29	45.0± 6.1b	83.0a	7.2	78	0.99	65
22	52.0± 2.6a	92.6c	7.0	92	1.14	103
30	44.2± 4.7b	79.6a	8.3	82	1.26	87
19	42.4± 3.2b	80.6a	7.8	95	1.13	89
24	49.0± 4.7ab	89.8bc	5.7	59	1.01	70
Mean	46.1± 2.2	85.7	7.1		1.10	
CV (%)	10.6	27.7	37.2		41.6	

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.11 Plant height (cm) of field pea lines at the onset of the tolerance trial at Holetta, Ethiopia.

Test Entry	Plant Height (cm)	
	To be infested	Not to be infested
05	15.2a*	16.2a
27	12.9bc	13.7bc
29	15.0ab	13.5bc
22	10.9c	11.5c
30	12.8c	12.2bc
19	13.0bc	14.1ab
24	11.9c	12.6bc
Mean	13.1	13.4
CV (%)	12.6	13.9

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.12 Tolerance component of resistance of field pea entries to pea aphid at Holetta, Ethiopia in 2000.

Entry Code #	Plant Growth, cm		% Uninfested plants
	Uninfested	Infested	
05	70.0±10.0ab*	18.6±4.1b	27.5b
27	68.0±7.9ab	18.3±1.9b	27.4b
29	68.2±9.9ab	27.6±3.7a	41.5a
22	63.0±5.0b	16.1±3.7b	26.1b
30	70.8±8.7ab	24.8±5.4a	35.9ab
19	76.4±9.0a	26.4±7.6a	34.6ab
24	66.4±6.7ab	18.2±1.7b	28.6b
Mean	68.0±1.7	21.4±3.4	31.7
CV (%)	14.3	21.7	28.2

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.13 Tolerance component of resistance of field pea entries to pea aphid at Holetta, Ethiopia.

Entry Code #	No. leaf infested	% Uninfested	Fresh plant weight (g) infested	% Uninfested	Dry plant weight (g) infested	% Uninfested
05	14.4c*	43.7bc	0.43de	25.6b	0.04c	27.8b
27	14.0c	39.2c	0.59de	29.0b	0.06c	35.6ab
29	30.6a	80.1a	1.55a	54.2a	0.16a	58.5a
22	21.4bc	59.8abc	0.59de	30.5b	0.06c	32.1b
30	25.0ab	69.5a	1.36ab	36.4ab	0.13ab	38.6ab
19	24.8ab	62.4ab	1.00bc	28.0b	0.11b	34.1b
24	28.8ab	60.7ab	0.94cd	25.4b	0.14ab	34.6b
Mean	22.7	59.4	0.92	32.7	0.10	37.3
CV (%)	25.6	27.2	31.7	45.5	33.8	43.7

* Means within column followed by the same letter are not significantly different at P = 0.005 (LSD)

Table 3.14 Component of resistance responses of field pea lines to *Acyrthosiphon pisum* at Bloemfontein, South Africa.

Entry Code #	Antibiosis (nymph/adult)	Antixenosis (adult/plant)	Tolerance (% of uninfested plant height)
05	31.6	8.9	69.2
27	24.8	6.7	81.6
29	21.8	6.6	63.2
22	42.2	11.6	76.9
30	24.0	9.4	69.4
19	24.6	7.7	70.3
24	20.2	8.4	75.0
Mean	27.0	8.5	72.2

Table 3.15 Normalized indices and overall resistance index (RI) based on components of resistance to *Acyrthosiphon pisum* in seven field pea lines at Bloemfontein, South Africa.

Entry Code #	Normalized indices			
	Antibiosis (x)	Antixenosis (y)	Tolerance (z)	PRI*
05	0.75	0.77	0.84	2.06
27	0.59	0.58	1.00	2.92
29	0.52	0.57	0.77	4.38
22	1.00	1.00	0.94	1.06
30	0.59	0.81	0.85	2.46
19	0.58	0.66	0.82	3.04
24	0.48	0.72	0.92	3.14

PRI = 1/(xyz) (Inayatullah *et al.* 1990, Webster, Starks & Burton 1987); indices calculated using x, y and z indices.

Table 3.16 Antibiosis, antixenosis and tolerance components of resistance of field pea lines to *Acyrthosiphon pisum* at Holetta, Ethiopia.

Entry Code #	Antibiosis (nymph/adult)	Antixenosis (adult/plant)	Tolerance (% of uninfested plant height)
05	74.9	4.9	27.5
27	77.4	6.7	27.4
29	93.6	5.3	41.5
22	86.7	7.1	26.1
30	76.6	7.6	35.9
19	88.1	7.5	34.6
24	95.4	9.0	27.4
Mean	84.7	6.9	31.7

Table 3.17 Normalized indices and overall resistance index (RI) based on components of resistance to *Acyrthosiphon pisum* in eight field pea lines at Holetta, Ethiopia.

Entry Code #	Normalized indices			
	Antibiosis (x)	Antixenosis (y)	Tolerance (z)	PRI*
05	0.78	0.54	0.66	3.6
27	0.81	0.74	0.66	2.5
29	0.98	0.59	1.00	1.7
22	0.91	0.79	0.63	2.2
30	0.80	0.84	0.86	1.7
19	0.92	0.83	0.83	1.6
24	1.00	1.00	0.66	1.5

*PRI = 1/(xyz) (Inayatullah *et al.* 1990, Webster *et al.* 1987); indices calculated using x, y and z indices.

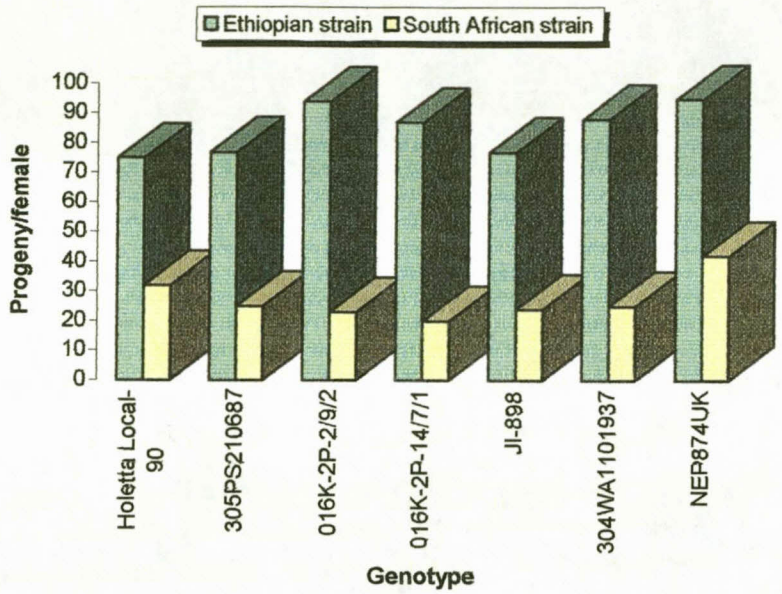

Figure 3.1. The number of nymphs produced per *Acyrthosiphon pisum* strains on seven field pea lines.

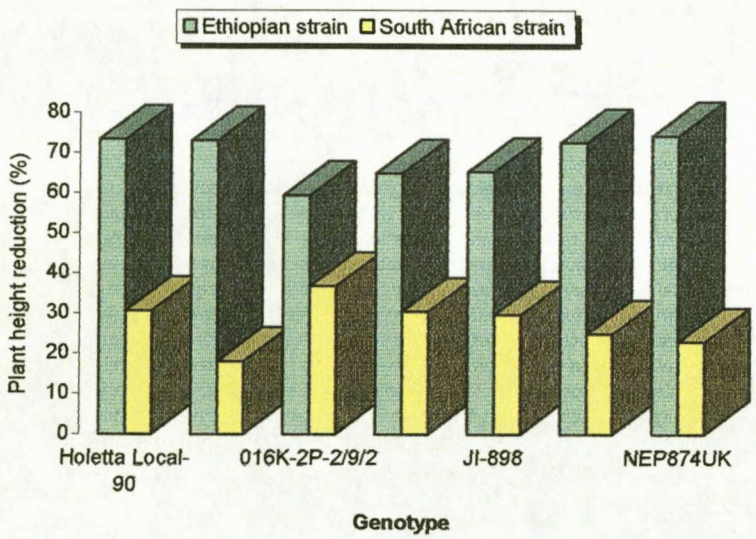

Figure 3.2. Plant height reduction (%) of seven field pea lines after 3 weeks infestation with the two strains of *Acyrthosiphon pisum*.

CHAPTER 4

Relative susceptibilities of field pea (*Pisum sativum* L.) genotypes to ascochyta blight caused by *Mycosphaerella pinodes*

Abstract

Ascochyta blight of field pea is prevalent in Ethiopia causing significant losses to crops annually. The best means of controlling the disease is by planting resistant genotypes. The susceptibility of 27 and three field pea genotypes originating from Ethiopia and South Africa respectively, to three isolates of *Mycosphaerella pinodes* from three geographic locations in Ethiopia (Denbi, Kulumsa and Holetta), were investigated by means of artificial inoculations in the glasshouse. Of the 6 isolates, 3 were selected based on the relative virulence by leaf disc bio-assay test. The severity of disease symptoms was recorded at 7, 14 and 21 days after inoculation and rated on a scale from 0 (no symptoms) to 5 (extensive lesions). Significant genotype isolate interactions were evident for all scoring dates. None of the genotypes were totally immune to the disease but low susceptibility to all three isolates was displayed by the South African cv. Oregon Sugar Pod II. The cv's. Green Feast, Sugar Queen and line 304WA1101973 were moderately susceptible while the remaining genotypes ranged from moderately susceptible to highly susceptible. The most widely grown cultivars in Ethiopia, Mohanderfer and G22763-2C, were as susceptible as Holetta Local, while the cv. Markos was moderately resistant to isolates from Holetta and Kulumsa. An analysis of variance indicated that the contribution of genotype x isolate interactions to disease severity was very low indicating that no pathotypes/races were represented by the three *M. pinodes* isolates despite the Denbi isolate being slightly more virulent than the Holetta and Kulumsa isolates.

Keywords: Resistance, *Pisum sativum*, Field pea, *Mycosphaerella pinodes*, ascochyta blight, virulence.

Introduction

Field pea (*Pisum sativum* L.) is the second most important food legume crop in the world after beans (*Phaseolus* spp.) (FAO 1999) and is an alternative crop for rotation with cereals, potatoes and oil crops in Ethiopia. In 1999, about 6 million hectares of field pea were cultivated worldwide yielding 10.5 million metric tons (FAO 2000). Despite the importance of the crop, most farmers in developing countries are still growing unimproved land races or local cultivars with small yields averaging less than 600 kg/ha (FAO 1999). The yield and quality of this important crop are significantly affected by numerous diseases that, depending on weather conditions, host susceptibility, and pathogen virulence, may cause severe losses.

Although fungi, bacteria, viruses and nematodes cause diseases of field pea, the crop is attacked by more than twenty fungal pathogens (Hagedorn 1985). Ascochyta blight, powdery, downy mildew, septoria blotch, damping-off and fusarium root-rot are the most serious fungal diseases (Barbetti & Brown 1993). Gorfu and Beshir (1994) reported 15 fungal pathogens affecting field pea in Ethiopia of which ascochyta blight and powdery mildew are the most widespread and destructive.

The ascochyta disease complex is caused by three fungi: *Ascochyta pisi*, *Mycosphaerella pinodes* (the perfect stage of *Ascochyta pinodes*) and *Phoma medicaginis* var. *pinodella*. *M. pinodes* is the most destructive pathogen of field pea throughout the world, particularly in France (Tivoli and Lemarchand 1992, Tivoli, Béasse, Lemarchand & Masson 1996), UK (Nasir & Hoppe 1991), Australia (Bretag, Keane & Price 1995), Canada (Warkentin, Rashid & Xue 1996) USA (Kraft 1991), and Ethiopia (Gorfu & Beshir 1994). It

infects pea seedlings as they emerge causing girdling which results in lodging (Ryan, Staunton & Cassidy 1984).

M. pinodes also colonizes maturing pods, from which the fungus invades developing seeds. Heavily infected seeds are 'stained' by the fungal mycelium while unstained seeds may also harbour the pathogen. The pathogen overwinters in soil and on crop debris (Sheridan 1973), and can develop rapidly during periods of wet weather and moderate temperatures. Typically, the first sign of infection is the appearance of small blue-black spots on aerial organs. The spots enlarge quickly under favorable environmental conditions, coalescing to form necrotic lesions and leading to premature senescence (Kerling 1949). Infection and sporulation starts at the base of the plant and progresses upward (Roger & Tivoli 1996). Healthy leaves may be infected either by pycnidiospores or ascospores carried in rain drops and dispersed by wind.

Garry, Tivoli, Jeuffroy & Cithavel (1996) demonstrated that ascochyta blight alters carbohydrate metabolism, protein remobilization and free amino acid translocation from hulls and leaves. These workers also noted that the disease reduced carbohydrate and nitrogen content in seeds, and that high disease severity affected the carbohydrate nitrogen ratio. The disease causes severe decreases in yield and seed quality (Allard, Bill & Touraud. 1992). Yield reductions of up to 70% have been recorded in severe cases (Ali, Nitschke, Dube, Krause & Cameron 1978, Lawyer 1984).

M. pinodes infects most pea crops in Australia, contributing to yield losses of 10 to 20% which in some years can result in a total crop loss on individual farms (Bretag *et al.* 1995). In France, yield losses can attain 40% in diseased plots (Tivoli *et al.* 1996) while in the USA, Sell & Aakre (1993) estimated that moderate to severe infections could reduce

yield by 20 to 50 %. Loss assessment studies at two locations in Ethiopia revealed a mean yield loss of 36.8% (range: 25 - 59 %) at Holetta, while at Denbi 24.2% (range: 10 - 40 %) was measured (IAR 1997).

Control measures aimed at limiting the development of the disease are based on repeated protectant sprays of fungicides applied at 10-15 d intervals from flowering to pre-harvest, regardless of the risk of infection (Warkentin *et al.* 1996, Roger, Tivoli & Hubber 1999). Currently, no genetic resistance/tolerance to *M. pinodes* has been developed in commercial pea cultivars (Kraft, Dunne, Goulden & Armstrong 1998) and no single gene for resistance has been found. Among 2936 accessions of *Pisum sativum* screened under field conditions, only five accessions were found to be resistant to *M. pinodes*.

The possibility of physiological specialization within *M. pinodes* makes it difficult to develop resistant cultivars. Previous studies have shown that the population of *M. pinodes* is highly variable and that it can be divided into pathotypes that differ markedly in their virulence on leaves of different P*isum* species or pea lines and cultivars (Nasir & Hoppe 1991). Clulow, Matthews & Lewis (1991) divided 50 single conidium-derived isolates into 6 pathotypes using 15 pea cultivars. Consequently, it may be necessary to incorporate several resistance genes for a cultivar to exhibit field resistance to *M. pinodes*. Several workers (Ali *et al.* 1978, Bretag 1989, Nasir & Hoppe 1997, Barangar & Tivoli 1998, Wroth 1998, Wroth & Khan 1999) have reported blight resistance in pea to *M. pinodes*. Clulow (1989) reported that several wild accessions of *P. sativum* were resistant in a controlled environment.

A few studies have been completed on the use of fungicides and host resistance in management of ascochyta blight. However, under present circumstances, when host resistance is unstable and the effectiveness of chemical control is low, integrated disease

management is the best approach. Reddy & Singh (1990) studied the potential for integration of host plant resistance and a limited number of foliar sprays and found that two sprays of chlorothalonil on a moderately resistant cultivar at seedling and early podding stages were most cost effective. Therefore, varietal resistance offers an opportunity to reduce unnecessary fungicide sprays. The objective of the present study was to evaluate cultivars and breeding lines of field pea for resistance to isolates of *M. pinodes* for possible use in an integrated disease management strategy.

Materials and Methods

Isolation of the pathogen. M. pinodes was isolated from diseased leaves and stems of various winter cultivars of field pea (mainly Mohanderfer and Tegegnech) at senescence. Collections were made from central and southeastern pea-growing areas of Ethiopia. Small portions of diseased tissues were surface sterilized (1 min in 96% ethanol, 3 min in a 3.5% NaOCl solution, and 30 s in 96% ethanol) and aseptically plated onto corn meal agar (CMA) amended with streptomycin (0.3 ml/L) in 90 mm petri dishes and incubated at 20 ± 1^0C.

Isolates were transferred to Coon's medium (4g maltose, 2g KNO_3, 1.2g $MgSO_4$, 2.7g KH_2PO_4 and 20g agar) and incubated for 14 days to obtain pycnidiospores. Six single spore isolates from four locations were obtained by streaking a suspension of spores onto water agar (1.5%) and transferring germinating cells to Coons agar.

Influence of temperature and culture media. The influence of temperature and culture media on mycelial growth of 6 isolates of *M. pinodes* was investigated. Three culture media, namely oatmeal, Coon's and potato dextrose agar (PDA) and five temperature regimes were used. The prepared media were dispensed into petri dishes (20 ml of medium per 9-cm-diameter dish). Mycelial plugs, 5 mm diameter, cut from the margin of an actively growing

culture on Coon's agar were placed in the center of the dishes. Cultures were then incubated in continuous darkness at 15, 20, 25, 30, and 35^{0}C. Linear mycelial growth of each isolate, on Coon's, oatmeal and PDA media, at different temperatures was determined in three replicate plates by measuring two radii of the colony every 3 days over a 15-day period using a digital caliper.

Leaf disc bio-assays. The relative virulence of the six isolates collected from pea fields in Ethiopia were evaluated on 14-day-old pea seedlings using a detached leaf bioassay. This was to select few virulent isolates to assess the susceptibility of field pea genotypes. Field pea plants (cv. Mohanderfer) were grown in plastic pots filled with sterilized soil/peat mixture in a greenhouse under natural daylight conditions. After two weeks, when the leaflets of the 3rd and 4th nodes were fully expanded, leaves of the same age were carefully removed from the plants and placed in petri dishes, the bases of which were lined with moist filter paper.

Spore suspensions were obtained by culturing the fungus on oatmeal agar at 20±1^{0}C (12-h photoperiod) for 14 days. Sterile distilled water was added to the 14-d-old cultures and the surface of the colony was rubbed with a glass rod. The spore suspension was filtered through four layers of cheesecloth to remove the mycelium. The spore concentration was determined with a haemacytometer and adjusted to 1x 10^5 spores per ml (Nasir & Hoppe 1997) with sterile water.

Eight leaves (4 per plate) were inoculated by placing 5µl of the spore suspension (1x 10^5 spores ml^{-1}) using a micropipette on each leaf. Control leaves were inoculated with equal amount of sterilized distilled water. The petri dishes were incubated at 20±1^{0}C. After six days, each leaf was assessed by measuring the area of necrotic lesion using digital caliper.

Glasshouse inoculations. Seven seeds of each of 30 pea genotypes (Table 4.1), including the susceptible control (Gorfu 2000), were grown in 1 liter plastic pots filled with steam sterilized, 1:1 v/v (volume ratio) mixture of soil and peat moss. Seedlings were thinned after emergence to five per pot and plants were maintained in an air-conditioned glasshouse (24/16^{0}C: day/night temperature) under natural daylight conditions until they were 4 weeks old. The experiment was arranged as a split-plot with three replications, each genotype representing the main plot and isolates the subplots. Treatments were represented by three pots with five seedlings per pot.

One drop (0.02 ml) of Tween 20 was added to a spore suspension prepared as described above and applied to 4-week-old plants with a hand-held multi-purpose atomizer. Control plants were sprayed with sterile distilled water supplemented with 0.2 % Tween. Pots were placed in a growth chamber at 20^{0}C with 16h: 8h (light:dark) photoperiod and a relative humidity of 75-85%. Day length was held to 16 h by using fluorescent and incandescent lighting.

Disease assessment and analysis. Seven, 14 and 21 days after inoculation, symptoms on each genotype were classified into six disease severity categories: 0= no infection; 1= <5% leaf lesions, but no stem lesions; 2= 5-10% leaf lesions, but no stem lesions; 3= 5-10% leaf and stem lesions; 4= 11-25% leaf and stem lesions, and dieback; and 5= > 25% leaf and stem lesions, and dieback. Mean values were calculated for the variables and the data were subjected to an analysis of variance (ANOVA) using the software package MSTAT-C (1990). Differences between isolates, genotypes and their interactions were also tested by ANOVA and means were compared using Duncan's Multiple Range Test.

Results

The results of leaf disc bio-assays showed that all the isolates were pathogenic on cv. Mohanderfer, producing necrotic lesions on detached leaves. The growth of *M. pinodes* in susceptible leaf material resulted in large areas of necrotic tissue at the point of inoculation. There was a clear variation in response within *M. pinodes* isolates in their ability to produce necrotic lesions. Based on these results, three relatively virulent isolates were identified to assess the susceptibility of 30 field pea genotypes.

Symptoms of ascochyta blight were observed 7 days after inoculation on all field pea genotypes inoculated in the greenhouse but no infection was observed in cv. Oregon Sugar Pod II for Holetta and Kulumsa isolates, Green Feast for the Kulumsa isolate and control plants. The ANOVA showed that the effects of genotype, isolate and genotype x isolate interaction were highly significant (Table 4.2). The initial disease severity was low on the first scoring (7 days after inoculation).

The mean virulence of isolates based on initial disease severity ranged from 0.1 for Oregon Sugar Pod II to 2.3 for Kyondo, but all isolates were weakly virulent on Green Feast, line 305ps210025, and 304WA1101937 and more virulent on NC 95 Haik, Markos, Kyondo and the susceptible check (Table 4.3). Although disease severity on all genotypes were low value, the two breeding lines and the two South African cultivars were the most resistant to all isolates. The most susceptible reactions occurred in Markos, line EXDZ and 061K-2P-2/9/2 with isolate Holetta, Mohanderfer, NC 95 Haik and Adi with Kulumsa isolate (Table 4.3).

At two weeks after inoculation significant differences were also observed between field pea genotypes, pathogen isolates and their interaction. Although genotype isolate interactions were significant for disease severity, their contribution to total variation was

much lower than that of genotypes and isolates separately (4.8%) (Table 4.4). The mean square for genotypes was higher than that for isolates, indicating that there was more variation between field pea genotypes than between fungal isolates.

The highest level of disease severity was observed two weeks after inoculation (Table 4.5) on the susceptible check, and the lowest on Green Feast and Oregon Sugar Pod II. The reaction of the susceptible check was similar for all isolates. Cv. NC95 Haik, and lines G22763x305PS210736-2, KFP-103, and 305PS210025 did not differ significantly ($P < 0.05$). On the 0-5 scale, the range of mean score in disease severity ratings two weeks after inoculation was 1.2 to 4.3 for Denbi isolate, 1.2 to 4.2 Holetta and 1.0 to 4.5 for Kulumsa (Table 4.5). Genotypes classified as resistant had an infection rate of 2 or less. There were three genotypes (305PS210025, Green Feast and Oregon Sugar Pod II) with a disease severity rating of 2 or lower. The commonly grown field pea cultivars in Ethiopia were found to be susceptible to all *M. pinodes* isolates, with Markos the only exception.

The highest disease severity was observed on Milky (4.2), Adi (4.1) EXDZ (4.2) and 305PS210900 (4.2) for Denbi isolate, Mohanderfer for Holetta (4.2) and Kulumsa (4.5) while Hassabe (4.3), JI-898 (4.2) and Kyondo (4.2) for Kulumsa isolate. In resistant reactions (categories 0-2), colonization was restricted to a few leaf areas, giving rise to variable numbers of necrotic flecks. In susceptible interactions (categories 3-5) the pathogen spread extensively within the host tissue and resulted in desiccation of the lower leaves.

The highest level of disease severity was observed after three weeks of inoculation (Table 4.7) on most genotypes. Results suggested that only one entry scored < 2, and four entries were selected as moderately resistant with ratings between 2.1 to 3.0. Twenty-five of the entries were found to be susceptible or highly susceptible (3.1 to 5.0). The analysis of

disease severity values showed significant differences among genotypes, isolates and genotype x isolate interactions (Table 4.6). However, the mean squares for genotypes were very high compared with those of the isolates indicating that more variation was present among genotypes than among pathogen isolates (Table 4.6).

The reaction of field pea seedlings 21 days after inoculation with *M. pinodes* can be separated into three distinct groups. These groups include: plants with little disease development (resistant); plants that were severely diseased with lower leaves desiccated and that died by week three (susceptible); and plants with extensive lesion development (intermediate). Based on disease severity ratings, Oregon Sugar Pod II was rated resistant; Green Feast, Sugar Queen, 304WA1101937 and NUR74BxFilby were rated moderately resistant while the remaining cultivars were rated susceptible or highly susceptible. All improved Ethiopian cultivars showed higher disease severities compared to the South African cultivars, the scores being in a range of 3.2 to 4.7 and 1.9 to 2.7, respectively. The disease in general progressed more slowly in Markos than in any other Ethiopian variety and most of the entries showed mean severity values similar to the susceptible check.

The most resistant genotypes to Holetta and Kulumsa isolates were Oregon Sugar Pod II and 304WA1101937. The reaction of the susceptible genotype was the same to all isolates. However, averaged over 30 genotypes, Holetta and Kulumsa isolates were relatively less aggressive than Denbi (Table 4.7).

The combined disease severity for *M. pinodes* pooled over all three scoring dates, on 30 field pea genotypes and three isolates are given in Table 4.8. A similar trend was evident, i.e genotypes, isolates and their interactions differed significantly in reaction to *M. pinodes* averaged over all scoring dates. The mean severity scores values across all scoring dates for

genotypes ranged from 1.1 for cv. Oregon Sugar Pod II to 3.6 for cv. Mohanderfer. That for isolates ranged from 3.0 for Denbi and 2.8 for Holetta and Kulumsa (Table 4.9).

Discussion

In the present study, the 30 genotypes, including the susceptible check, showed high variability in their reaction to the three isolates of *M. pinodes*. The ranking of genotypes did not however change significantly over a period of three weeks. Disease symptoms were similar but slightly more definitive 14 and 21 days after inoculation compared with 7 days after inoculation. The results also show the existence of local isolates with some variation in aggressiveness. The information derived from this study is fundamental for the development of field pea cultivars resistant to *M. pinodes* under Ethiopian conditions.

The cultivars, Milky, Mohanderfer, NC95 Haik, G22763-2C, Hassabe and Adi were highly susceptible to all isolates despite having previously been exposed to selection for resistance in a routine breeding program. Cultivars adapted to Ethiopia were found to be susceptible to all isolates, suggesting a possible increase in the virulence of the pathogen population over the last 5-10 years. It is believed that emergence of new virulent forms in a pathogen population is not only an outcome of genetic recombination, but could also be the effect of direct selection for virulence in the pathogen population (Leonard 1987).

The contribution of genotype-isolate interactions to variation was very low and differences in pathogenicity were not attributable to differences in the virulence of the isolates, but rather to resistance/susceptibility differences in the genotypes. This does not provide definite evidence for the existence of pathotypes of *M. pinodes*, suggestions for which have been said to be arbitrary and subjective (Knappe & Hoppe 1995). Pathotype groups differing in aggressiveness were however identified among isolates of *M. pinodes* in

previous reports (Ali *et al.* 1978, Clulow *et al.* 1991, Nasir & Hoppe 1991). Although pathotype groups could not be clearly identified, differences in aggressiveness were shown using 30 field pea genotypes and this may help to identify isolates useful for screening field pea lines for resistance to *M.* pinodes. Our results are consistent with results obtained by Onfroy, Tivoli, Corbière & Bouznad (1999) and Nasir & Hoppe (1997) who demonstrated that the contribution of genotype x isolate interactions was very low.

Genotype x isolate interactions was evident in numerous cases. For example, cv. Markos and line KFP–103 were moderately resistant to Holetta and Kulumsa isolates but showed higher susceptibility to Denbi isolate (Table 4.7). The cv. Oregon Sugar Pod II was resistant to all isolates, as expected. The majority of genotypes included in the study were not resistant, thus the trend toward increased damage with increasing crop age may reflect the tendency of most genotypes to succumb to prolonged ascochyta blight infection. Susceptibility to disease is likely to vary during plant development from the seedling stage through to the onset of fruiting (Lawyer 1984).

M. pinodes, is known to be a highly variable fungus and breakdown of resistance to the pathogen is a frequent phenomenon (Nasir & Hoppe 1991). The local breeding effort to develop blight-resistant field pea cultivars may therefore need to incorporate broad-spectrum or multigenic resistance in order to be successful. In other words, a clear understanding of the pathogenic variability of *M. pinodes* populations would be useful in breeding field pea cultivars with durable resistance. Because a limited number of isolates were used, it was not possible to assess variability critically. The recognition of increased virulence in the pathogen is important because resistant cultivars are a primary control method for ascochyta blight in areas where the disease is important. To develop cultivars with adequate levels of

resistance, disease evaluations should be made with highly virulent populations. The sum of mean square values for genotype x isolate interactions (Table 4.6) was only about 2.6% of the sum square values due to genotypes and isolates (97.4%). This indicated that the contribution of genotype x isolate interactions to variability was very low and does not suggest the existence of distinct races in *M. pinodes*.

The pea aphid population density (Chapter 2) and ascochyta blight severity scores were negatively correlated to both Ethiopian (r = -0.290) and South African (r = -0.054) strains and neither of these were significant (P = 0.800 and P = 0.346, respectively). The low correlation between disease infection and pea aphid infestation scores indicates that no pea genotypes have resistance to both pests. Most of the lines having resistance to pea aphid are highly susceptible to ascochyta blight caused by *M. pinodes*.

The present study clearly showed that there were differences between responses of field pea genotypes inoculated with isolates of *M. pinodes* under controlled conditions. Field pea cvs. currently under cultivation, and most of the breeding lines, are susceptible to ascochyta blight. Nevertheless, combining the use of existing resistant genotypes with good agricultural practices supplemented by the use of seeds produced in disease-free areas or treated with fungicides, can increase and stabilize yields of field pea in Ethiopia.

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Table 4.1 Varieties/lines and reference numbers for *Pisum sativum* genotypes screened for resistance to three isolates of *Mycosphaerella pinodes*.

Variety / line	Origin	Status	Reference number
Adi	Ethiopia	Released variety	1
Green Feast	South Africa	Released variety	2
G22763-2C	Ethiopia	Released variety	3
Hassabe	Ethiopia	Released variety	4
Holetta Local-90	Ethiopia	Released variety	5
Markos	Ethiopia	Released variety	6
Milky	Ethiopia	Released variety	7
Mohanderfer	Ethiopia	Released variety	8
NC 95 Haik	Ethiopia	Released variety	9
Oregon Sugar Pod II	South Africa	Released variety	10
Sugar Queen	South Africa	Released variety	11
EXDZ	Ethiopia	Breeding line	12
G22763X305ps210736-2	Ethiopia	Breeding line	13
HI-7	Ethiopia	Breeding line	14
HI-21	Ethiopia	Breeding line	15
JI-91	Ethiopia	Breeding line	16
JI-116	Ethiopia	Breeding line	17
JI-898	Ethiopia	Breeding line	18
KFP-103	Ethiopia	Breeding line	19
Kyondo	Ethiopia	Breeding line	20
NEP 874 UK	Ethiopia	Breeding line	21
Nur 74B x Filby	Ethiopia	Breeding line	22
304 WA 1101937	Ethiopia	Breeding line	23
305PS 210025	Ethiopia	Breeding line	24
305PS 210572	Ethiopia	Breeding line	25
305PS 210687	Ethiopia	Breeding line	26
305PS 210900	Ethiopia	Breeding line	27
061K-2P-2/9/2	Ethiopia	Breeding line	28
061K-2P-14/7/1	Ethiopia	Breeding line	29
Holetta-Local (Susceptible)	Ethiopia	Farmer's variety	30

Table 4.2 Results of analysis of variance of data for disease severity of 30 pea genotypes, one week after inoculation with *Mycosphaerella pinodes* isolates in growth chamber.

Source of variation	Degrees of freedom	Mean square
Genotype	29	2.59***
Isolate	2	1.08**
Genotype x Isolate	58	0.31**
Error	120	0.19

*** Significant at P = 0.001, ** Significant at P = 0.01,

Table 4.3 Mean initial disease severity (0-5 scale) one week after inoculation on 30 pea genotypes inoculated with 3 isolates of *Mycosphaerella pinodes* in growth chamber, Bloemfontein, 2001.

Genotype	Isolate			Mean
	Denbi	Holetta	Kulumsa	
Adi	2.0	1.3	2.2	1.8
Green Feast	0.3	0.3	0.0	0.2
G22763-2C	1.7	1.0	1.7	1.4
Hassabe	1.8	1.7	2.3	1.9
Holetta Local-90	1.5	1.2	1.7	1.4
Markos	2.5	2.0	1.7	2.1
Milky	1.8	2.0	1.0	1.6
Mohanderfer	1.7	1.8	2.5	2.0
NC 95 Haik	2.2	1.7	2.5	2.1
Oregon Sugar Pod II	0.3	0.0	0.0	0.1
Sugar Queen	2.3	2.0	1.7	2.0
EXDZ	2.3	2.0	1.3	1.9
G22763X305ps210736-2	1.7	1.3	1.3	1.4
HI-7	1.7	2.0	1.5	1.7
HI-21	1.3	1.3	1.0	1.2
JI-91	1.7	1.8	1.0	1.5
JI-116	2.0	1.7	1.0	1.6
JI-898	1.3	1.3	1.7	1.4
KFP-103	1.7	1.5	1.7	1.6
Kyondo	2.2	2.2	2.5	2.3
NEP 874 UK	1.8	1.8	1.2	1.6
Nur 74B x Filby	2.0	1.7	2.0	1.9
304 WA 1101937	0.7	1.0	0.3	0.7
305PS 210025	0.3	0.7	1.0	0.7
305PS 210572	1.5	1.0	1.2	1.2
305PS 210687	2.0	2.0	1.7	1.9
305PS 210900	2.2	1.5	1.2	1.6
061K-2P-2/9/2	1.3	1.3	1.3	1.3
061K-2P-14/7/1	1.3	1.3	1.2	1.3
Holetta-Local (Susceptible)	2.3	2.3	2.0	2.2
Mean	1.6	1.5	1.4	

LSD (5%) = 0.70
CV (%) = 28.5

Table 4.4 Analysis of variance of data for disease severity (0-5 scale) of 30 pea genotypes, two weeks after inoculation with *Mycosphaerella pinodes* isolates in growth chamber.

Source of variation	Degrees of freedom	Mean square
Genotype	29	5.26***
Isolate	2	0.88**
Genotype x Isolate	58	0.31**
Error	120	0.19

*** Significant at P = 0.001, ** Significant at P = 0.01.

Table 4.5 Mean initial disease severity (0-5 scale) two weeks post inoculation on 30 pea genotypes inoculated with 3 isolates of *Mycosphaerella pinodes* in growth chamber, Bloemfontein, 2001.

Genotype	Isolate			Mean
	Denbi	Holetta	Kulumsa	
Adi	4.1	3.2	4.0	3.8
Green Feast	1.5	1.2	1.0	1.2
G22763-2C	3.2	3.2	3.0	3.1
Hassabe	3.5	3.8	4.3	3.9
Holetta Local-90	3.2	3.8	2.8	3.3
Markos	2.3	2.5	2.3	2.4
Milky	4.2	3.5	3.3	3.7
Mohanderfer	3.7	4.2	4.5	4.1
NC 95 Haik	3.8	3.7	3.7	3.7
Oregon Sugar Pod II	1.2	1.3	1.0	1.2
Sugar Queen	3.2	2.7	2.7	2.8
EXDZ	4.2	3.7	3.5	3.8
G22763X305ps210736-2	3.3	3.3	3.2	3.3
HI-7	3.5	3.2	3.3	3.3
HI-21	3.2	3.0	2.7	2.9
JI-91	3.3	3.7	3.3	3.4
JI-116	3.0	3.0	2.7	2.9
JI-898	3.2	3.3	4.2	3.6
KFP-103	2.2	2.3	2.3	2.3
Kyondo	3.7	3.2	4.2	3.7
NEP 874 UK	3.3	3.2	2.7	3.1
Nur 74B x Filby	3.5	2.8	3.0	3.1
304 WA 1101937	2.7	2.2	1.8	2.2
305PS 210025	1.8	1.7	2.0	1.8
305PS 210572	2.7	2.8	2.5	2.7
305PS 210687	3.5	3.8	3.0	3.4
305PS 210900	4.2	3.2	3.0	3.4
061K-2P-2/9/2	3.5	3.3	3.2	3.3
061K-2P-14/7/1	3.7	3.5	3.2	3.4
Holetta-Local (Susceptible)	4.3	4.2	4.3	4.3
Mean	3.2	3.1	3.0	

LSD (5%) = 0.70
CV (%) = 13.9

Table 4.6 Results of analysis of variance of data for disease severity of 30 pea genotypes, three week after inoculation with *Mycosphaerella pinodes* isolates in growth chamber.

Source of variation	Degrees of freedom	Mean square
Genotype	29	6.20***
Isolate	2	0.96***
Genotype x Isolate	58	0.19**
Error	120	0.10

*** Significant at P = 0.001, ** Significant at P = 0.01.

Table 4.7 Mean initial disease severity (0-5 scale) three weeks post inoculation on 30 pea genotypes inoculated with 3 isolates of *Mycosphaerella pinodes* in growth chamber, Bloemfontein, 2001.

Genotype	Isolate			Mean
	Denbi	Holetta	Kulumsa	
Adi	4.7	4.5	4.5	4.5
Green Feast	2.3	2.2	2.0	2.2
G22763-2C	4.3	4.2	4.0	4.2
Hassabe	4.3	4.7	5.0	4.7
Holetta Local-90	4.2	4.7	4.0	4.3
Markos	3.7	3.0	3.0	3.2
Milky	4.8	4.3	4.5	4.5
Mohanderfer	4.3	4.7	5.0	4.7
NC 95 Haik	4.8	4.5	4.0	4.4
Oregon Sugar Pod II	2.2	1.7	1.8	1.9
Sugar Queen	2.8	2.7	2.7	2.7
EXDZ	4.7	4.3	4.3	4.4
G22763X305ps210736-2	3.2	3.0	4.2	3.4
HI-7	4.0	3.8	4.0	3.9
HI-21	4.0	3.7	4.0	3.9
JI-91	4.3	4.7	4.5	4.5
JI-116	4.8	4.7	4.5	4.7
JI-898	4.3	4.5	4.8	4.5
KFP-103	3.7	3.0	3.0	3.2
Kyondo	4.8	4.3	4.8	4.7
NEP 874 UK	4.2	4.0	4.2	4.1
Nur 74B x Filby	3.3	3.0	2.8	3.0
304 WA 1101937	2.7	2.0	2.0	2.2
305PS 210025	4.0	3.8	4.0	3.9
305PS 210572	3.8	3.7	3.5	3.7
305PS 210687	4.8	4.8	4.7	4.8
305PS 210900	4.7	4.3	4.2	4.4
061K-2P-2/9/2	4.2	3.8	4.0	4.0
061K-2P-14/7/1	4.2	4.0	3.8	4.0
Holetta-Local (Susceptible)	5.0	4.7	5.0	4.9
Mean	4.1	3.9	3.9	3.9

LSD (5%) = 0.50,
CV (%) = 7.9

Table 4.8 Combined analysis of variance for disease severity of 30 field pea genotypes inoculation with three *Mycosphaerella pinodes* isolates in a growth chamber.

Source of variation	Degrees of freedom	Mean square
Genotype	29	3.82***
Isolate	2	0.96***
Genotype x Isolate	58	0.16***
Error	120	0.07

*** Significant at P = 0.001.

Table 4.9 Mean (averaged over all scoring dates) initial disease severity (0-5 scale) on 30 pea genotypes inoculated with 3 isolates of *Mycosphaerella pinodes* in growth chamber.

Genotype	Isolate			Mean
	Denbi	Holetta	Kulumsa	
Adi	3.7	3.0	3.5	3.4
Green Feast	1.4	1.2	1.0	1.2
G22763-2C	3.1	2.8	2.9	2.9
Hassabe	3.2	3.4	3.9	3.5
Holetta Local-90	2.9	3.2	2.8	3.0
Markos	3.3	2.8	2.6	2.9
Milky	3.6	3.3	2.9	3.3
Mohanderfer	3.2	3.5	4.0	3.6
NC 95 Haik	3.6	3.3	3.4	3.4
Oregon Sugar Pod II	1.2	1.0	0.9	1.1
Sugar Queen	2.8	2.4	2.3	2.5
EXDZ	3.7	3.3	3.1	3.4
G22763X305ps210736-2	2.7	2.5	2.9	2.7
HI-7	3.1	3.0	2.9	3.0
HI-21	2.8	2.7	2.6	2.7
JI-91	3.1	3.4	2.9	3.1
JI-116	3.3	3.1	2.7	3.0
JI-898	2.9	3.1	3.6	3.2
KFP-103	2.5	2.3	2.3	2.4
Kyondo	3.6	3.2	3.8	3.5
NEP 874 UK	3.1	3.0	2.7	2.9
Nur 74B x Filby	2.9	2.5	2.6	2.7
304 WA 1101937	2.0	1.7	1.4	1.7
305PS 210025	2.1	2.1	2.3	2.1
305PS 210572	2.7	2.5	2.4	2.5
305PS 210687	3.4	3.6	3.1	3.4
305PS 210900	3.7	3.0	2.8	3.1
061K-2P-2/9/2	3.0	2.8	2.8	2.9
061K-2P-14/7/1	3.1	2.9	2.7	2.9
Holetta-Local (Susceptible)	3.9	3.7	3.8	3.8
Mean	3.0	2.8	2.8	

LSD (5%) = 0.43
CV (%) = 9.4

CHAPTER 5

An evaluation of *Hippodamia variegata* (Coleoptera: Coccinellidae) and an entomopathogenic fungus, *Beauveria bassiana*, for biological control of pea aphid

Abstract

The effect of a predator, *Hippodamia variegata* (Coleoptera: Coccinellidae), and an entomopathogenic fungus, *Beauveria bassiana,* in regulating pea aphid populations on field pea was evaluated in field cages in Bloemfontein, South Africa during 2001. Caged plants were inoculated with the same initial density of aphids (one aphid/plant), and the predator or fungus biocontrol agent was introduced after the population had reached 10-12 aphids/plant. After the 3rd week of coccinellid introduction aphid incidence was significantly lower in the *H. variegata* treatments than in the *Beauveria* and infested control treatments. Coccinellid-treated plots had fewer aphids (52/sampling unit) in comparison to fungus-treated (102/ sampling unit) and infested control (108/ sampling unit) plots. The results indicated that the use of *H. variegata,* the predominant predator in alfalfa fields, could comprehensively control pea aphid in a confined environment. *H. variegata* applied at one coccinellid adult per 200 pea aphids (1:200) provided the best aphid control and an optimum grain yield of 2000 kg/ha. Yield in the fungus treated plots was 1830 kg/ha and was comparable to the infested, untreated plots of 1700 kg/ha. Use of *H. variegata* as a control agent increased yields by 18.0%.

Key words: Coccinellidae, Entomopathogenic fungi, pea aphid, biological control.

Introduction

The pea aphid, *Acyrthosiphon pisum* (Harris), is an economically important pest of field pea, *Pisum sativum* L., in Ethiopia and elsewhere. Though the aphid transmits numerous virus diseases (Damsteegt, Stone, Russo, Luster, Gildow & Smith 1999), its main damage is done directly by feeding when favorable climatic conditions and/or lack of natural enemies permit the species to build up to enormous populations. Estimated losses in field pea due to direct feeding damage in Ethiopia range as high as 59% (Ali 1999).

Chemical means for control of pea aphid are available (Biddle, Blood-Smyth, Tabot & Smyth 1994, Bhatnagar 1996, Ali 1997), but such treatments may have adverse environmental effects and require annual expenditure for control. Moreover, it is usually uneconomical for the small-scale farmers to attempt to reduce pest populations with pesticide applications. Biological control would provide environmentally safe, long-lasting, and therefore potentially inexpensive, control. Pea aphid is attacked by a number of natural enemies including entomopathogenic fungi (mostly of the order Entomophorales), various arthropod predators, and hymenopterous parasitoids.

Capitalizing on the antagonism between pests and natural enemies in order to reduce the need to use pesticides to control species of economic importance is at the core of IPM philosophy. Progress and achievements in biological control of insect pests of tropical crops clearly show that the use of predators, parasitoids and pathogens for pest control, either by introduction from other sources or by exploiting those already in the area, has proved effective and economically beneficial (Sankaram 1986).

Perhaps the most effective natural control agents of explosive pest populations in annual crops are entomopathogens (Hajek & Leger 1994). Entomopathogenic fungi

constitute a unique group of insect pathogens. The most widely used in biological control of insect pests are the Deuteromycetes, of which *Beauveria bassiana* and *Metarhizium anisopliae* are the two most prominent members. *B. bassiana* has been tested in the laboratory and field against numerous pests in various cropping systems, *e.g.* European corn borer (Feng, Carruthers, Larkin & Roberts 1988), Russian wheat aphid (Vandenberg 1996), coffee berry borer (De La Rosa, Alatorre, Barrera & Toriello 2000, Grimm 2001), sugarcane stalk borer (Legaspi, Poprawski & Legaspi 2000), whiteflies (Wraight, Carruthers, Jaronski, Bradley, Garza & Galaini-Wraight 2000), as well as for effects on nontarget organisms (Goettel, Poprawski, Vandenberg, Li & Roberts 1990). Some success with *B. bassiana* and *M. anisopliae* against pea aphid has been reported in the USA (Pickering & Gutierrez 1991). Boverin, a commercial preparation of *B. bassiana* has been used extensively in the ex-Soviet Union for the control of potato beetle, and Metaquino, a commercial product of *M. anisopliae,* has been used for the control of sugarcane pests in Brazil (Ferron 1981).

Coccinellids play an important role in aphid population regulation and are known to have the strongest impact of all aphidophagus insects (Hodek, 1970; Kring, Gilstrap & Michels 1985, Hodek & Honek 1996). Kring *et al.* (1985) demonstrated that the introduction of two *Hippodamia* species into field cages excluding all other natural enemies resulted in reduced *Schizaphis graminum* (Aphididae) populations on grain sorghum, in light and moderate infestations.

Many studies have examined the impact of these natural enemies one at a time, demonstrating their potential under certain conditions, to keep aphid population below damaging levels (Chambers, Sunderland, Stacey & Wyatt 1986, Gutierrez, Hagen & Ellis 1990, Wratten & Powell 1991, Wraight, Poprawski, Meyer & Peairs 1993). However, there

have been fewer studies examining the control potential of these natural enemies under field conditions. Owing to the importance of aphids as pests of peas, and the reported abundance of natural enemies in many countries, this experiment was undertaken to evaluate the potential of a coccinellid and a fungus in regulating aphid populations in pea fields.

Materials and Methods

Experimental plots were located at the agronomy experimental site, on the west campus of the Free State University, Bloemfontein (1351 m a.s.l.; $29^0$06'S, $26^0$18'E), South Africa. One square meter of 'Mohanderfer' field pea stand (4 rows, 1 m in length) was established, making a total of 16 plots. Each plot was then covered using gauze cages supported by metal frames. An alley (2 m wide) and kept clean of vegetation, separated the blocks. The cages served to exclude general phytophagous insects, and in the case of the control, to compensate for possible variations in plant growth caused by the screen cages. A randomized complete block design was used with four replications. The total numbers of plants in each cage were estimated by counting the number in two rows per cage, obtaining an average per row, then multiplying by 4. Aphids were introduced at a rate of one aphid per plant, by random selection from greenhouse colonies when the crops, excluding the uninfested controls, were 1½ months old. The plots were mist-irrigated three times per week. Irrigation was omitted if rain had fallen since the last application of water. The experimental plots were planted on 21 August 2001 and harvested on 18 December 2001.

Insect culture. The pea aphids used in this experiment were collected from alfalfa, *Medicago sativa* L. near Bethlehem, South Africa. They were maintained in a greenhouse on potted pea plants, cv. 'Mohanderfer' at 20±1^{0}C, 60-70% RH, and a 16-h (16L:8D) photoperiod. These aphids were also used as prey for the coccinellids.

Pathogen culture. An isolate of the entomopathogenic fungus *B. bassiana* (PPRI 6370) derived from *Lysathia* sp. (Coleoptera: Chrysomelidae) obtained from the ARC Plant Protection Research Institute in Pretoria was cultured on sabouraud dextrose agar (40g dextrose, 10g peptin, 15g agar, 10g yeast extract and 0.3 ml streptomycin) in petri dishes (9-cm diameter). The culture was then incubated at 27 ± 1^{0}C with 12-h photoperiod. After 12-15 days, the conidia were harvested by scraping off the contents of each petri dish with a sterile bacteriological loop. The concentration of conidiospores in the suspension was determined with a haemocytometer and adjusted to 10^{6} conidia per ml (James, Brenda, Croft & Lighthart 1995) with sterilized distilled water. The conidiospore suspension was applied directly on aphid-infested field pea in cages with a small hand-help sprayer. Viability of the conidia was checked by means of a germination test (Legaspi *et al.* 2000) prior to the experiment and found to be > 97%.

Insect rearing. The *Hippodamia variegata* colony was initiated using adult beetles collected from alfalfa fields at the Glen Agriculture Experimental Station, 25 km north-east of Bloemfontein in September 2001. These were placed in glass vials plugged with cotton wool at one end and monitored daily for egg production. Eggs obtained from these beetles were incubated at 24^{0}C and the larvae reared to maturity. The pairing adults formed the breeding nucleus from which all further experimental materials were derived. As eggs were generally laid on the cotton wool plugs of the vials, they were merely transferred on the plug to a clean vial ready for incubation and further experimentation. This procedure facilitated transfer and avoided unnecessary handling of eggs.

Larvae, owing to their cannibalistic disposition, were reared individually in smaller vials. These were arranged standing upright in suitably drilled wooden racks. Adult beetles

were satisfactorily maintained in larger vials. The active stages (adults and larvae) of the insects were fed on pea aphids established on pea leaves and this food resource was replenished every morning, after the vials has been cleaned, until pupation of the mature larvae. Care was taken to assure that excess food was always available, since both development and fecundity are apparently influenced by food shortage. After sclerotization freshly emerged adults were placed in clean glass vials with aphid infested pea plants to begin the cycle again, or were used in the experiment.

Introduction of *H. variegata* was made when the pea aphids reached a level of 10-12 individuals per plant. Coccinellids were introduced into the cages at the ratio of one coccinellid per 200 pea aphids to ensure establishment inside cages. The experiment was arranged in a randomized complete block design with four treatments and four replications as follows:

A. *H. variegata* adults were used at the rate of one coccinellid per 200 pea aphids.

B. Conidial suspension of Entomopathogenic fungus (at a rate of 137 ml/plot at a concentration of 1.4×10^{12} conidia/ha).

CI: Control I; untreated aphid-infested cages.

CII: Control II; uninfested, untreated cages, these cages were kept aphid-free by applying Pirimor 50% WP (0.5% concentration) every two weeks.

Densities of aphids and *H. variegata* larvae and adults in each cage were estimated every week. Sampling was carried out by placing white cardboard (0.25m x 0.25m) randomly on the soil surface inside each cage, then gently shaking the plants above the cardboard and counting the aphids landing on the cardboard surface. Aphid mortality caused by entomopathogenic fungi was assessed 14 days after inoculation and thereafter every seven

days. Dead aphids were inspected for the presence of the fungal growth (white mycelium and spores) and recorded as death caused by fungus (mycosed). Those not showing the fungus were placed on moist tissue paper and incubated at 24^0C and monitored for fungal development. Plant height, number of pod/plant, number of seed/pod and weight of 100 seeds were recorded for each treatment. Harvesting in 1 m^2 was to assess biomass and grain yields. The biomass and grain yields were determined from four rows and then converted per hectare basis (10,000 y/x; y = plot yields, x = Plot size). Yield loss was determined in relation to the uninfested control and expressed as a %.

Data analysis. Analysis of variance was performed using MSTAT-C for each parameter considered. Least Significant Difference (LSD) test was used to separate means if F-test showed significant effects.

Results

Analysis of variance shows that the overall mean number of aphids was significantly different ($p<0.05$) between the treatments (Table 5.1). Coccinellid-treated cages supported significantly lower aphid numbers (52.3 aphids/0.25m^2 cardboard) than the fungus-treated (102.5 aphids/0.25m^2 cardboard) and infested control treatments (108.3 aphids/0.25m^2 cardboard). There was no significant difference between fungus-treated and infested control plots in aphid population density (Table 5.1).

Weekly pea aphid population trends averaged across replicated treatments for live aphids in the presence and absence of natural enemies are presented in Figure 5.1. The aphid density was similar in week 1 between all treatments. By the third sampling, aphid numbers declined in the coccinellid cages, whereas in the infested untreated control the population was 4 times as large as at the beginning of the treatments. At this point aphid numbers began

declining at a faster rate in the coccinellid-treated cages than in fungus-treated and infested untreated control cages. In the absence of *H. variegata*, densities of pea aphids increased rapidly in number after week 2 (Figure 5.1). At week 3, the number of aphids was twice as great in the fungus treatment than in the predator treatment. The pea aphid population trend in fungus-treated and infested control treatments was similar. The mean number of aphids treated with the fungus and infested control was also similar throughout the season reaching a maximum of 171/cardboard. Two weeks after introduction of the fungus, a mean of 11.4% of the aphids were infected, compared to only 2.6% in week 4. The highest incidence of mycosis by *Beauveria bassiana* on pea aphid was 14.3% and occurred 3 weeks after conidia application. Overall, however, the treatment had little effect on aphid survival indices. Few aphids were killed initially, but the fungus failed to spread in any of the plots after week 3, probably because of the prolonged dry, sunny weather during November and December. In contrast *H. variegata* quickly established themselves in the plots with a maximum larval count of 18/cardboard. In the treatments larvae started appearing towards the third week and they limited the potential increase of aphid numbers inside the cages.

There were no significant variations ($P>0.05$) in plant height, seeds/pod and 100-seed weight between treatments (Table 5.2). Pods/plant were significantly lower ($P<0.05$) in infested control and fungus-treated plots. However, no difference was observed between predator and uninfested treatments. Grain and biological yields significantly increased ($p<0.05$) in the aphid-free as well as predator treatments (Table 5.3). The biomass yields of infested untreated control (5390 kg/ha) and the fungus treated plots (5870 kg/ha) were significantly different from the aphid-free plots (6630 kg/ha). Predator-treated plots produced slightly lower aerial biomass yield than aphid free plots. However, differences were not

significant (P>0.05). With regard to grain yields, the aphid-free plots yielded significantly more (P<0.05) than predator and fungus–treated plots (Table 5.3). Yields obtained in the coccinellid-treated (2180 kg/ha) plots were significantly higher (P<0.05) than fungus (1830 kg/ha) and infested, untreated control (1700 kg/ha) plots. The infested, untreated control plot sustained the highest yield loss (22.0 %), followed by the *Beauveria*-treated plot (16.0%) (Table 5.3). Predator-treated plots recorded the lowest yield loss (8.3%). From these figures it is clear that yield loss in *Beauveria*-treated plots was double that in predator-treated plots.

Discussion

The results presented here provide evidence, from field trials, that justify the artificial establishment of a predator within populations of pea aphid damaging field pea. In a small plot field experiment, pea aphid population density analysis showed that the release of the predator exerted a suppressive effect on pea aphid population growth on field pea when compared with plots which did not receive the treatments (Table 5.1.). This study is only one of a few that evaluated natural enemies (predator and pathogen) on pea aphid in the field.

When *H. variegata* adults were released into high densities of aphids on caged field pea, there were significant differences between the number of aphids in treated and untreated cages, and these differences were also reflected in the yield of the crop. Losses in pods/plant, biomass and grain yields were caused by aphids feeding in the infested, untreated control. In the absence of *H. variegata*, peas became colonized by aphids and suffered losses of up to 22% in grain weight. These results demonstrate the feasibility of mass-rearing and releasing indigenous coccinellids to control aphid populations in field plots of field pea and it can be regarded as acceptable proof that a natural enemy can reduce the abundance of a pest species. Decreased pest levels and increased yield, relative to an infested control, reflected the true

efficiency of the locally prevalent coccinellids under largely natural conditions. The only unnatural situation in this study was the restraint in free dispersal of the pest and the natural enemy due to caging. Exclusion cages have proved effective in identifying the role of predators in the control of a number of different pests, particularly aphids (El Hag 1992). A complex of predators, each responding to changes in aphid density have also been reported to control pea aphid on alfalfa (Frazer, Gilbert, Nealis & Raworth 1981).

In general fungal infection on pea aphids was low (2.6 % at week 4). It is believed that the expression of virulence was affected by various factors, of which the most important were the strain of the fungus used, dynamics of the pest, timing of spraying and the prevailing environmental conditions after application. Moreover, the degree of exposure of the target depends largely on the possibility that the pest will come in contact with the microbial pesticides. Roberts, Fuxa, Gaugler, Goettel, Jaques & Maddor (1991) have thoroughly debated the influence of these factors on the effectiveness of entomopathogenic fungi. It is also highly likely that the extended exposure of the experimental plots to sunlight had a negative affect on the success of the fungal agent. In this regard Edgington, Segura, De La Rosa & Williams (2000) observed that spores of *Beauveria bassiana* were completely inactivated by direct exposure to sunlight after a period of 60 minutes.

B. bassiana had no effect on pea aphid numbers in the field cages in this study. After introduction into pea aphid populations in the field, the fungus only established for a brief period and failed to spread. These results are in agreement with results obtained by James *et al.* (1995) who demonstrated that despite high persistence of *B. bassiana*, the pathogen had no effect on pea aphid numbers in field cages. Similarly, Dorschner, Feng & Baird (1991) showed up to 100% mortality in the hop aphid, *Phorodon humuli* (Aphididae) (Schrank)

when exposed to *B. bassiana* in the laboratory, but that the fungus failed to control aphids in the field. This result could be ascribed to either the aphids not receiving a sufficient dose in the field or to weather conditions not being sufficiently suitable for infection to occur. Germination of *B. bassiana* is greatly reduced at relative humidity levels less than 95% (Doberski 1981). The low relative humidity in the field during this study was possibly the major factor that prevented infection because a relative humidity of < 50% results in no germination of *B. bassiana* conidia (Teng 1962). Despite significant mortality in laboratory bioassays, the failure of fungal mycoinsecticides to induce field epizootics is commonly reported (Dorschner *et al.* 1991, James *et al.* 1995). Many failures are likely the result of unfavorable relative humidity and temperature conditions in the field, relative to those in the laboratory.

An important difference between laboratory bioassays and field experiments is that bioassays assess individuals and many field trials assess populations. Pea aphids have a very short generation time of approximately 7 days at 20^0C (Campbell & Mackauer 1975) and a very high fecundity rate. It takes 3-6 days for the fungus to kill an adult aphid, and that same adult may continue to reproduce during the incubation period. This time delay in mycosis development may impede the short-term efficacy of *B. bassiana* for aphids. To improve control with *B. bassiana*, more needs to be known about the environmental conditions necessary for the pathogen to be effective. For one, improving formulations to increase infection rates may help to reduce dependence on favorable weather conditions. Several frequent applications may be required for pests such as aphids that have a short generation time and high fecundity, particularly if the pest is not highly mobile which might lead to less rates of conidia pick-up and decrease the risk to infection. The time duration between sprays

should be less than the generation time of the pest. In general, the study shows that pea aphid infestation on field pea resulted in significant reduction of grain and biological yields in the absence of any control measure. These observations seem to suggest that use of *Hippodamia variegata* as biological control can effectively reduce pea aphid populations and minimize yield loss.

The use of *Beauveria* should be incorporated into an IPM strategy in such a way that the moderate levels of mortality caused by the fungus are complimented by cultural and other biological control agents such as predators. It is concluded that the release of indigenous coccinellids can provide augmentative control of pea aphids and minimize grain yield loss.

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Table 5.1 Average number (mean of five counts) of pea aphids per 0.25m^2 cardboard on field pea treated with *Hippodamia variegata* and *Beauveria bassiana* at Bloemfontein, South Africa in 2001.

Treatment	Aphid density/0.25 m^2 board*
CI (infested control)	118.3a
B (*B. bassiana*)	102.5a
A (*H. variegata*)	52.3b
CV (%)	11.99

* Means followed by the same letter are not significantly different at P = 0.005 (LSD).

Table 5.2 Average yield components of field pea treated with *Hippodamia variegata* and *Beauveria bassiana* at Bloemfontein, South Africa in 2001.

Treatment	Yield components*			
	Pods/plant*	Seeds/pod	Plant height (cm)	100- seed weight (g)
CI (infested control)	11.5b	4.0	182	15.2
CII (uninfested control)	17.5a	3.9	181	14.7
A (*H. variegata*)	15.8a	3.7	179	16.5
B (*B. bassiana*)	12.7b	3.9	176	15.5
CV (%)	10.04	11.98	7.12	9.50

* Means followed by the same letter are not significantly different at P = 0.005 (LSD).

Table 5.3 Yields of field pea and yield loss due to pea aphid infestation in plants treated with *Hippodamia variegata* and *Beauveria bassiana* at Bloemfontein, South Africa in 2001.

Treatment	Yield (kg/ha)		Yield loss (%)
	Biological*	Grain	
CI (infested control)	5390c	1700c	22.0
CII (uninfested control)	6630a	2180a	-
A (*H. variegata*)	6370ab	2000b	8.3
B (*B. bassiana*)	5870bc	1830c	16.0
CV (%)	6.48	7.14	

*Means followed by the same letter are not significantly different at P = 0.005 (LSD).

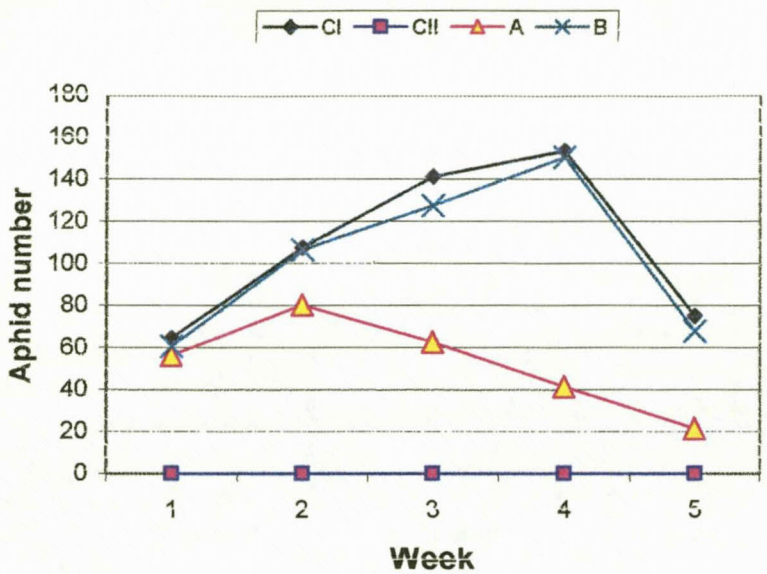

Figure 5.1 Average number of aphids per 0.25 m^2 cardboard treated with *Hippodamia variegata* and *Beauveria bassiana* at Bloemfontein, South Africa in 2001. CI = infested control, CII = uninfested control, A = treated with *H. variegata*, B = treated with *B. bassiana.*

CHAPTER 6

The effect of mixed cropping of *Pisum sativum* L. on *Acyrthosiphon pisum* (Harris) infestation and ascochyta blight infection in Ethiopia

Abstract

The effect of mixed cropping field pea (*P. sativum*) with faba bean (*Vicia faba*), wheat (*Triticum aestivum*) and Ethiopian mustard (*Brassica carinata*) on ascochyta blight incidence and the population dynamics of pea aphid and its natural enemies was studied during the 2000 growing season. The experiment was conducted at three locations, in a randomized block design involving seven treatments, each replicated four times. Data collected were ascochyta blight severity, population densities of *Acyrthosiphon pisum* and its natural enemies, biomass (aerial plant weight) and grain yields. Field pea intercropped with mustard sustained less disease and *A. pisum* incidence compared with field pea planted in a monoculture system at all three locations. Results across three locations showed that the field pea plus Ethiopian mustard intercropping was advantageous in terms of overall grain yield, land equivalent ratio (LER) and economic return in the subsistence farming system.

Key words: *Acyrthosiphon pisum*, ascochyta blight, mixed cropping, field pea.

Introduction

The cultivation of more than one crop in a field, commonly referred to as mixed cropping or intercropping, is a popular cultural practice in virtually all subsistence agriculture. The system is a widespread practice in many parts of the world (Seshu Reddy 1990), although it varies from one location to another depending on the dominant crops. Reasons for practicing intercropping include risk aversion through crop diversification, compensation for total crop failure in the event of natural disasters such as droughts and pest outbreaks, and to produce a variety of crops required by farmers. Research findings have shown that intercropping also has other merits. These include reduction in pest population numbers of one or more of the crops included in the cropping mixture (Andow 1991, Alghali 1993), reduction in the severity of some diseases (Kikoka, Katunzi & Teri 1989, Tesfamichael & Reddy 1996, Teferi 1997, Gorfu 1999) and increased total yields. Intercropping has been reported to promote natural biological control in certain crop combinations (Altieri & Liebman 1986). Intercropping may also result in a reduction in the amount of pesticide needed for the effective management of pests (Alghali 1993), thereby reducing both production costs and the negative effects of pesticides on the environment. In general, the system ensures greater yield stability over seasons, higher yield per unit area of land, better use of resources and maintenance of soil fertility.

A mixture of cereals with grain legumes has been the most preferred combination by small-scale farmers in the tropics. In Uganda, cowpea and sorghum are commonly grown in mixtures. The ratio of the two crops depends on the farmer's main crop (Obuo, Osiru & Adipala 1997). In the savanna regions of West Africa, farmers normally grow cowpea in mixtures with either sorghum, millet or groundnut, or sometimes all three crops together

(Norman, Simmons & Hays 1982). However, most studies on cowpea intercropping studies on pest populations have used sorghum or maize as cereal combination crops (Bottenberg, Tamo & Singh 1998). The intercropping of faba bean (*Vicia faba*) with spring wheat and pea with barley was studied in Denmark and Nepal (Andersen, Haahr, Jensen & Sandfaer 1983, Jensen 1986, Subedi 1998). Agriculture in Ethiopia is largely of a subsistence nature and some farmers practice mixed cropping. A survey carried out at various locations indicated that farmers grow field pea in mixture with faba bean (Beyene, Nigatu & Woldemariam 1994). Chickpea and lentil are also mixed-cropped with sorghum and maize (Eshete & Beniwal 1988).

Reports from elsewhere have shown that some intercropping patterns reduce the incidence of infestation by pests (Risch, Andow & Altieri 1983, Weiss, Schatz, Gardner & Nead 1994, Alghali 1993, Olufemi, Pitan & Odebiyi 2001). However, other reports have indicated that intercropping only has a limited effect on pest populations. Bottenberg *et al.* (1998) reported that mixed cropping only had a limited potential as a means of controlling cowpea pests. Certain reports have also indicated that some intercropping patterns may actually lead to higher incidence of crop damage by insect pests (Dissemond & Hindrof 1989) and diseases (Dissemond & Hindorf 1989, Kikoka *et al.* 1989, Allen 1990). Therefore, in view of these diverse findings, the merits of a given intercropping system should be based on the pest and disease situation at a target locality. In Ethiopia, there is no information available regarding the effect of intercropping on pea aphid infestation in field pea.

The aim of this study was to assess the potential of a legume:legume:non-legume intercropping system as a cultural pest management strategy against pea aphid and ascochyta

blight in Ethiopia. The focus of this investigation was an evaluation of the influence of a single field pea cultivar mixed with faba bean, Ethiopian mustard and wheat on the incidence of pests and/or disease relative to field pea cultivated in a monoculture system.

Materials and Methods

Field experiments were carried out at Holetta ($38^0$31'E $09^0$03'N, 2400 masl), Denbi ($38^0$59'E $8^0$46'N, 1900 m a.s.l.) and Kulumsa ($39^0$11'E $8^0$03'N, 2200 m a.s.l.) during the winter of 2000. The meteorological data, soil surface physio-chemical characteristics, and physiographic features of the experimental fields are presented in Tables 6.1. Normal rainfall from the seedling to harvest stages characterized the 2000 field season (refer to Appendix 1). The experiment was designed as a randomized complete block with four replications at each location for one season. Pure stands of field pea, faba bean, wheat and Ethiopian mustard (*Brassica carinata* Braun.), respectively, were established at a seedling rate of 150, 200, 150 and 15 kg ha^{-1} kg. There were three mixed stands: field pea + faba bean at 112.5:50 kg ha^{-1}, field pea + wheat (112.5:37.5 kg ha^{-1}) and field pea + Ethiopian mustard (112.5:3.75 kg ha^{-1}). The plot size was 4 x 5 m and seeds of all crops were sowed at the same time without any row arrangements (mixed intercropping) in late June. This planting date coincides with the practice of local farmers in Ethiopia. Experimental plots were fertilized with Diammonium phosphate (DAP) at a rate of 100 kg (containing 18 kg N and 46 kg P_2O_5) per hectare at sowing and incorporated into the soil. Weeds were controlled by hand weeding and no pesticides were applied. Field pea varieties used were Mohanderfer at Kulumsa and Denbi and Tegegnech at Holetta. This selection was based on the variety suitable to respective environmental conditions. Varieties of Ethiopian mustard and wheat were Yellow Dodolla and Galema respectively. Two faba bean varieties were also used, CS-

20DK (Holetta) and NC-58 (Denbi and Kulumsa). Tillage at all locations was conventional with moldboard plowing followed by a disk harrowing. The experiments were sown on 19 June at Holetta and 26 June at Denbi and Kulumsa.

After the first appearance of aphids in field pea plots, (early August), data on any aphid populations were recorded for 10 randomly selected plants in each plot throughout the season. Whole plants were examined during the first few weeks of the season, but as plants grew larger only the terminal 20-30 cm of the stem tips were inspected. Parasitised aphids were also noted on the same 10 plants. Intensive scrutiny for predators (mainly coccinellids and syrphids) were conducted and individuals counted on a plot basis. Each plot was rated for blight severity using a 0 to 5 scale (Roger, Tivoli & Huber 1999). Disease ratings commenced when the blight symptoms appeared and each plot was rated seven times during the season at weekly intervals. Measurements of plant stands at emergence and at harvest, biomass and seed weight, pods per plant, seeds per pod were made. Hundred seed weight for faba bean and field pea was determined by weighing 100 grains while thousand kernel weight was used for wheat by weighing 1000 grains from dried samples. Land equivalent ratio (LER) was calculated for pure and mixed stands. The index commonly used to evaluate an intercropping system is the Land Equivalent Ratio (LER) (Willy 1979), which is defined as:

$$LER = \sum (Xi / Yi)$$

where Xi is the yield of the crop I in the intercropping setup and Yi is the yield of crop I in the pure stand setup. The LER essentially compares productivity in intercropping with that in the monocrop, with high values of LER indicating the advantage of intercropping.

Values for the area under the disease progress curve (AUDPC) for each treatment were calculated using the formula of Shaner & Finney (1977):

$$AUDPC = \sum_{i=1}^{n} \left[\frac{(Y_{i+n1} + Y_1)}{2} \right] [X_{i+1} - X_1]$$

in which Yi = Ascochyta blight severity (per unit) at the ith observation, Xi = time (days) at the ith observation, and n = total number of observations.

An analysis of variance (ANOVA) was conducted using MSTAT-C (1990). Data from individual sites were analyzed separately. Yield components and grain yields of wheat, faba bean mustard and peas were analyzed. Differences between treatments were compared using the Least Significance Difference (LSD, P = 0.05) between means, if F-test showed significant effects. Net monitory returns were calculated by deducing the variable cost for seed from gross returns, based on the fact that other variables (*i.e.* fertilizer, labor, land preparation etc.) were similar for all the treatments.

Results

The field pea/mustard mixed crops were practically consistent in sustaining significantly lower incidence of ascochyta blight infection than the field pea monocrops in Holetta and Denbi in most of sampling dates recorded (Figures 6.1 and 6.2). The mixture also showed the lowest incidence of infection at Kulumsa (Figure 6.3). However, the difference among the treatments was not statistically significant ($P<0.05$), except at the fourth sampling date. These observations suggest that the presence of mustard in the mixed crop cultivation served to reduce the severity of ascochyta blight in these treatments. The ascochyta blight progress curve was also lowest for field pea/mustard mixtures and significantly different from the other intercrop treatments at Holetta and Denbi, but not at

Kulumsa. However, the degree of disease development on all treatments over the whole season was highest at Denbi, followed by Kulumsa, while the least was from Holetta. At all locations, disease incidence was the lowest in field pea mixed with mustard (Table 6.2). Of all the treatments, including the field pea monocultures, disease pressure was highest in field pea intercropped with faba bean and wheat.

Overall and at all three locations, incidence and size of aphid colonies were the highest in monoculture field pea than was the case in the other three mixtures (Table 6.3). At Holetta, average aphid densities per plant on monoculture field pea were significantly different ($P<0.05$) compared with the other mixed croppings. Mixed-pea treatments had similar mean number of aphids per plant. At Denbi there were significantly more aphids per plant in field pea plots than in mixtures with wheat and mustard. The numbers of *A. pisum* per plant was not affected by the cropping system at Kulumsa. However, the number of aphids per plant in the field pea/mustard mixture was smaller than compared to other treatments, but the differences were not significant. The pea aphid densities were different between the locations, with lower values for the cool, high rainfall site (Holetta), compared with the warm, mid-altitude sites (Denbi and Kulumsa respectively). In general, *A. pisum* was more numerous at Denbi and Kulumsa and peaked during September 15-20, *i.e.* at flowering time. This is an important period with regard to damage levels and grain yield (Table 6.3). *A. pisum* populations were about five times higher at Denbi and Kulumsa than at Holetta.

The cropping system had no impact on the degree of parasitization by *Aphidius* spp., (Brachonidae) which was not significantly different at all the experimental sites. The percentage of *A. pisum* parasitised by wasps varied between locations. There were more

parasitised aphids in field pea/faba bean mixtures at Holetta (9.0%) and Denbi (8.7%) than monoculture field pea plots, while it was 7.7% in field pea / mustard mixtures at Kulumsa. The population numbers of predators were too low to be assessed.

Results on agronomic parameters are presented in Table 6.4. The effect of mixed cropping on the yield components of field pea was inconsistent across sites. Yield components were slightly affected by mixed cropping treatments. The number of pods per plant and seeds per pod of field pea at harvest was not affected by the different treatments at Holetta and Kulumsa. However, at Denbi, field pea/mustard mixtures presented significantly lower pods per plant compared with other treatments, which was reflected in the grain yield at this site. At Kulumsa, none of the yield components were affected by mixed cropping.

Significant treatment effects were observed for aerial mass yield of field pea in all locations (Table 6.5). At Holetta, mixing field pea with faba bean (3450 kg ha-^{1}) and mustard (1525 kg ha-1) significantly reduced the biomass yield of field pea (4325 kg ha^{-1}). At Denbi, the biomass yield of field pea mixed with faba bean (2450 kg ha^{-1}) and wheat (2350 kg ha^{-1}) was more than that of the pure stand (2112 kg ha^{-1}). At Kulumsa, the biomass yield was higher compared with the other two sites, whilst the field pea mixed cropping with mustard had the lowest yield which was significantly different from the pure stand and field pea mixed with faba bean.

There were significant differences regarding the field pea grain yield at all the locations (Table 6.5), in particular reflecting reduced yield when mixed with mustard. The mean grain yield of field pea in pure stand was higher at Holetta (1640 kg ha^{-1}) than at Denbi (395 kg ha^{-1}) and Kulumsa (1231 kg ha^{-1}), whilst the field pea/mustard combination presented the maximum yield. In general, field pea grain yield at Denbi was lower than at

Holetta and Kulumsa due to a higher incidence of ascochyta blight and pea aphid damage. The field pea/wheat mixed intercrop yielded higher than the other two cropping arrangements in the study at Holetta and Kulumsa, and was significantly higher than field pea/mustard mixtures (Table 6.5). Generally, field pea in the field pea/wheat intercrops sustained lower yield losses than the field pea/faba bean or field pea/mustard mixtures. Mixed crops produced mean seed yields of 2108 kg ha^{-1}(ranging from 1354 to 3221 kg ha^{-1}), 1891 kg ha^{-1} (range of 911 to 3853 kg ha^{-1}), and 2046 kg ha^{-1} (range of 1432 to 3081 kg ha^{-1}) at Holetta, Denbi and Kulumsa respectively.

At all locations, the field pea suffered a great deal when mix cropped with mustard, as compared to faba bean and wheat. This might be attributed to increased competition for soil and water requirements. Mustard growth was tall and dense, which also resulted in shading, which in turn could possibly have contributed to reduced field pea yield in the mixture. However, the lower yield was compensated for by the yield of mustard when compared with other mixed crop arrangements.

The advantage gained from intercropping, compared with growing pure stands is often evaluated by calculating Land Equivalent Ratio (LER). If LER is higher than unity, then there is an advantage (increased biological efficiency) from intercropping when compared with growing pure stands. LER values calculated from grain yield was higher than unity in field pea/mustard mixed intercropping at all locations, whilst only a minor advantage may be obtained from mixed cropping of field pea/wheat at Kulumsa. Field pea intercropped with mustard gave higher total LERs of 1.72 at Holetta followed by Kulumsa (1.3) (Table 6.6).

In terms of economic return, Table 6.6 indicates that the highest net benefit of 10042, 12966 and 9431 birr ha^{-1} was obtained when field pea was mixed cropped with mustard at Holetta, Denbi and Kulumsa respectively (1 US$ = 8.50 birr on 20 April 2001). Monoculture field pea stands gave the lowest net benefit at Denbi and Kulumsa. Generally the trend was that the higher the LER value, the better the net benefit (Table 6.6). The additional advantages of mixed cropping biomass as livestock feed have not been considered in the economic analysis.

Discussion

Ascochyta blight incidence and aphid numbers decreased in mixed cropping field pea stands, when compared to pure field pea stands. This study confirms that increased crop diversity within field cultivation can result in fewer pests and diseases. The major benefit in this regard is reflected in foliar diseases. In mixtures of field pea with mustard, significantly lower ascochyta incidence was recorded at all the experimental sites with the exception of Kulumsa where a difference was noted towards the end of the season. The mechanism by which field pea/mustard intercropping reduced blight was not investigated in this study. However, it has been speculated that the presence of mustard in field pea/mustard mixtures, acts as a barrier to blight spread through wind and rain splash. Another possible reason for the observed differences could be due to shading and other microclimatic effects under field pea/mustard intercropping which could also cause a reduction in the infection and development efficiency of the pathogen (Pfleeger & Mundt 1998). Thus, field pea / mustard mixed crops buffer against blight incidence by delaying the development of the disease or reducing spore dissemination. Spores are wind borne enabling them to spread very rapidly over large areas. When they land on mustard leaves in the mixed cropping set-up, they fail to

reproduce which leads to a direct reduction of ascochyta blight inoculum and consequently a lower infection rate on the field pea plants. In line with these findings, Belay (1992) demonstrated that intercropping with maize reduced the spore dispersal capabilities of bean rust. Gorfu (1999) also illustrated that ascochyta blight severity was disproportionately but significantly reduced in field pea/faba bean mixed crops. Similarly Guar & Singh (1996) reported that chickpea intercropped with mustard had the lowest *Ascochyta rabiei* infection compared with barley, pea or lentil intercropping, but that yield was also greatly reduced. This phenomenon was generally corroborated by the results of this study when field pea and mustard were intercropped. This finding is in agreement with reports that intercropping pulses with non-pulse crops reduces the incidence of aphids in legumes (Coderre, Provencher & Champagne 1989, Ogenga-Latigo, Ampofo & Baliddawa.1992, Olufemi *et al.* 2001). Mittal (1997) also showed that disease incidence on lentils was less in mixtures with wheat than was the case in the pure crop. This system also has a benefit. The mustard plants acted as support structures onto which the field pea twined, thereby reducing the direct contact of the pea plants with the soil. Reducing the soil contact of the peas may also decrease the incidence of disease by providing a drier microclimate, which may improve the quality of the harvested pea seed (Weiss *et al.* 1994).

In general mixed cropping had some effect on pea aphid population sizes in that pea aphid colonies were smaller in mixtures, compared with pure field pea stands. While it is difficult to explain the manners in which aphids responded to increased crop diversity, it is well known that plant quality differences among crops can affect aphid population development. In this regard it is possible that differences in chemical or visual stimuli emanating from different crops could have played a role. Data from the mustard mixtures,

however, suggest that physical differences were important, since the dispersion of a 'short' pea variety among tall and dense mustard plants restricted aphid settling by mustard plants providing a protective barrier.

These findings differ from that by workers at ICRISAT (1980) who found that *Rhopalosiphum maidis* (Aphididae) populations on sorghum were not different in sorghum monoculture and polycultures of sorghum and pigeon pea. On the contrary, the observation by Helenius (1991) showed that peak densities of cereal aphids were 30-85% higher in mixtures of oats/faba bean than in the pure stand of oats. He attributed the main mechanism contributing to this to be the concentration of alate colonies in fewer tillers per unit area within the mixtures. In the present study, total aphid density per plant was much lower in the mixtures than in the field pea monoculture, especially in field pea/mustard mixtures. The results across three locations consistently showed that mixed cropping is preferable to cultivating field pea, as opposed to pure stands in terms of overall grain yield, economic advantage and LER. Thus intercropping seems to be desirable in order to meet dietary requirements and fodder supply. With regard to LER, Ghizaw (1994) reported a value of 1.31 for 3:1 ratio of field pea to faba bean at Holetta. In the present study, the corresponding values were 0.83 (Holetta), 0.79 (Denbi) and 0.89 (Kulumsa) respectively. The reported values are less than unity in this investigation.

The incidence of certain natural enemies, (coccinellids, syrphids) on field pea plants was not affected by mixed cropping. However, *Aphidius* spp. appeared to be attracted when field pea was mixed with faba bean at Holetta and Denbi. Helenius (1990) reported similar findings in that the population sizes of natural enemies of *Rhopalosiphum padi* (Aphididae) in oats / faba bean mixtures were not significantly affected by mixed cropping when

compared to oats monocropping. However, unlike the results presented here, Zhang (1990) showed that the number of natural enemies found in intercropped cotton was five times higher than that found on the cotton monocrop.

The lack of statistical significance (at p=0.05) for some of the parameters considered in this study may be attributed to the characteristically irregular pattern of distribution of plant damage under natural aphid infestation. Such a phenomenon is known to cause high variability. Nevertheless, a general pattern was observed whereby the intercropping of field pea with mustard reduced pea aphid incidence and damage on field pea. In this respect, therefore, the field pea/mustard mixed cropping arrangement was more effective combination. Besides this, there was an also additional harvest of mustard attained from the mixed crop set-up. This is an important value-added factor, particularly if one takes into consideration that most Ethiopian farmers are small-scale and resource poor. By developing farming systems that utilize low-cost cultural control practices, important contributions will be made towards increased yields and reduced crop losses due to insect pests and diseases.

The potential of mixed cropping, especially as a component of an integrated pest management system in Ethiopia is promising and deserves to be investigated further. This study presented some evidence that mixed cropping might provide some yield stability under adverse pest and disease conditions. Since this is largely a traditional practice, it need not suffer communication barriers as many new technologies tend to do. Of course, the technique has its drawbacks, with the major problem being that in mixed cropping system the different plants have a wide maturity range. For example, mustard is phenologically late maturing whereas field peas mature relatively early. This obviously has implications regarding pest and disease occurrence. Therefore the identification of suitable varieties and

the right proportion of seed rates for intercropping field pea with cereals and mustard should be a priority research area in order to reduce pest and disease incidence and increase productivity of the system.

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Table 6.1 Soil physio-chemical characteristics, altitude and meteorological data for the three experimental sites in Ethiopia used for field pea mixed cropping during the period 2000.

Characters	Holetta	Denbi	Kulumsa
Altitude ma.s.l.	2400	1900	2200
Mean max. temperature (^{0}C)	22.7	26.4	22.7
Mean min. temperature (^{0}C)	5.9	10.2	12.2
Mean annual rainfall (mm)	925	805	777
Long-term average	1077 (31 yrs)	871 (10 yrs)	788 (33 yrs)
Soil texture	Clay	Loam	Clay
Clay (%)	67.5	40.0	55.0
Sand (%)	20.0	30.0	26.2
Silt (%)	12.0	30.0	18.7
Organic Carbon (%)	1.48	1.99	2.30
Nitrogen (%)	0.12	0.13	0.14
Available P (ppm)	12.60	44.60	8.80
pH (H_2O)	4.04	6.11	5.81
Na (meg/100g)	0.69	0.132	0.112
K (meg/100g)	0.817	1.229	1.715
Ca (meg/100g)	11.192	22.504	23.088
Mg (meg/100g)	3.307	23.716	18.122

Table 6.2 Mean score (0 – 5 scale) and area under the disease progress curve (AUDPC) in percentage by days for ascocyta blight of field pea. Means within rows followed by the same letter are not significantly different at P=0.05 by least significant test. 1= field pea, 2 = faba bean, 3 = wheat, 4 = mustard

Treatment	Holetta		Denbi		Kulumsa	
	mean score	AUDPC	mean score	AUDPC	mean score	AUDPC
FP[1]	2.0a	477.7a	2.7a	2138.5a	2.8	1083.7
FP/FB[2]	1.8a	468.1a	2.7a	1238.5a	2.9	1510.7
FP/W[3]	1.8a	475.1a	2.5a	1955.6a	2.9	1575.9
FP/M[4]	1.5b	366.2b	2.2b	1032.5b	2.5	1080.2
CV (%)	4.6	5.1	5.0	10.1	10.9	15.5

Table 6.3 Effect of mixed intercropping on pea aphid population density (aphid/plant) on field pea (Fp) at the three experimental sites in Ethiopia during 2000. Means within respective rows, without letters in common, differ significantly at $p<0.05$ (LSD). (1 = faba bean, 2 = wheat and 3= mustard, Fp = field pea).

Sampling date	Intercropping pattern			
	Fp	Fp+Fb[1]	Fp+W[2]	Fp+M[3]
Holetta				
21/8	1.4	0.7	0.5	0.2
28/8	3.0	0.9	0.8	0.1
05/9	1.6	1.0	1.9	0.8
13/9	1.5	1.8	1.5	0.3
18/9	3.0	1.8	1.5	3.3
25/9	3.2	2.6	3.1	2.0
02/10	3.9	2.5	1.3	1.6
Mean	2.5a	1.6b	1.5b	1.4b
Denbi				
15/8	7.3	7.6	8.2	4.3
22/8	11.0a	12.8a	5.9b	5.2b
29/8	24.7a	19.5ab	14.8b	13.7b
07/9	9.9	12.1	21.2	8.9
13/9	8.8	5.6	5.3	3.8
19/9	20.9a	18.9a	5.3b	6.2b
26.9	16.9a	12.2ab	8.4b	5.9b
Mean	14.2a	12.7ab	9.7bc	7.1c
Kulumsa				
23/8	3.2	3.3	1.5	6.6
30/8	1.1	3.2	4.0	3.1
06/9	6.3	4.5	6.5	0.9
12/9	9.6	7.8	15.4	2.7
20/9	14.2a	16.1a	11.2b	5.7c
27/9	12.7a	10.8ab	15.1b	4.3c
04/10	10.0	12.2	12.9	11.6
Mean	8.2	8.3	9.5	5.0

Table 6.4 Effect of different mixed cropping combinations on the yield components of field pea (Fp), faba bean (Fb), mustard (M) and wheat (W) at Holetta, Denbi and Kulumsa, Ethiopia during 2000. Means within column, without letters in common, differ significantly at $p<0.05$ (LSD). (1 = days to flower, 2 = days to mature)

Treatment	Pod/plant	Seed/pod	DF[1] (day)	DM[2] (day)	Height (cm)	100-seed weight (g)
Holetta						
Fp	10.2	4.2	64.0b	138.0	179	20.0
Fp+Fb	10.3	4.1	64.2b	134.2	166	21.5
Fp+W	11.7	4.0	65.7a	133.7	175	22.0
Fp+M	7.4	3.9	64.0b	136.0	172	21.5
Mean	9.9	4.1	64.5	135.5	173	21.2
CV (%)	16.73	11.33	1.03	2.01	5.02	7.92
Denbi						
Fp	9.0a	3.6	62.5a	99.2	168	15.0
Fp+Fb	8.0ab	3.4	58.0b	98.2	164	14.7
Fp+W	7.6b	3.5	62.0a	99.0	169	14.0
Fp+M	6.0c	3.2	60.5ab	97.0	161	14.2
Mean	7.6	3.4	60.7	98.4	166	14.5
CV (%)	8.57	8.68	2.85	1.77	3.72	14.4

Table 6.4 continued...

Kulumsa

Fp	13.5	3.9	62.5	122.5	165	15.0
Fp+Fb	13.4	4.0	63.0	123.2	167	13.7
Fp+W	11.7	4.4	63.0	122.7	169	15.2
Fp+M	13.1	4.5	62.0	122.7	167	15.2
Mean	12.9	4.2	62.6	122.8	167	14.8
CV (%)	21.0	11.4	1.0	1.4	6.5	8.7

Table 6..5 Effect of different intercropping combinations on biomass and grain yields (kg/ha) of field pea, faba bean, wheat and Ethiopian mustard at Holetta, Denbi and Kulumsa, Ethiopia during 2000. Means within column, without letters in common, differ significantly at $p<0.05$ (LSD).

Crop treatment	Biomass Yield (kg ha^{-1})			Grain yield (kg ha^{-1})			
	Fp	Fb	W	Fp	Fb	W	M
Holetta							
Field pea pure stand (Fp)	4325a	-	-	1640a	-	-	
Faba bean pure stand (Fb)	-	3377a	-	-	1722a	-	
Wheat pure stand (W)	-	-	9887a	-	-	2010a	
Mustard pure stand (M)	-	-	-	-	-	-	3087a
Fp+Fb	3450b	1209b	-	1236b	118b	-	
Fp+M	1525c	-	-	496c	-		2725a
Fp+W	3637a	-	2062b	1337b	-	411b	

Table 6.5 Continued...

Denbi

Crop treatment	Biomass Yield (kg ha^{-1})			Grain yield (kg ha^{-1})			
	Fp	Fb	W	Fp	Fb	W	M
Field pea pure stand (Fp)	2112ab	-	-	395a	-	-	-
Faba bean pure stand (Fb)	-	7587a	-	-	3310a	-	-
Wheat pure stand (W)	-	-	8425a	-	-	3236a	-
Mustard pure stand (M)	-	-	-	-	-	-	3925a
Fp+Fb	2450a	2150b	-	211b	699b	-	-
Fp+M	1750b	-	-	91c	-	-	3762a
Fp+W	2350a	-	2425b	160bc	-	751b	-

Kulumsa

Crop treatment	Biomass Yield (kg ha^{-1})			Grain yield (kg ha^{-1})			
	Fp	Fb	W	Fp	Fb	W	M
Field pea pure stand (Fp)	5362a	-	-	1231a	-	-	-
Faba bean pure stand (Fb)	-	10,312a	-	-	5662a	-	-
Wheat pure stand (W)	-	-	6687a	-	-	2377a	-
Mustard pure stand (M)	-	-	-	-	-	-	3712a
Fp+Fb	4850a	1125b	-	930b	695b	-	-
Fp+M	3737b	-	-	917b	-	-	2162b
Fp+W	4512ab	-	437b	1126ab	-	306b	-

Table 6.6 Land Equivalent Ratio (LER) and net economic benefit (birr, 1US$ = 8.50 birr) of different intercropping patterns and in pure stand for field pea, faba bean, wheat and Ethiopian mustard based on the mean grain yield at Holetta, Denbi and Kulumsa, Ethiopia during 2000.

Holetta

Crop treatment	Fp	Fb	W	M	Total	Net benefit (birr ha^{-1})*
Field pea (Fp)	1.00 **a**	-	-	-	1.00	3530
Faba bean (Fb)	-	1.00 **a**	-	-	1.00	2728
Wheat (W)	-	-	1.00 **a**	-	1.00	3251
Mustard (M)	-	-	-	1.00 **a**	1.00	10750
Fp+Fb	0.76 **b**	0.07 **b**	-	-	0.83	2720
Fp+W	0.31**c**	-	0.20 **b**		0.51	3563
Fp+M	0.83**b**	-	-	0.89 **a**	1.72	10042

Denbi

Crop treatment	Fp	Fb	W	M	Total	Net benefit (birr ha^{-1})
Field pea (Fp)	1.00 **a**	-	-	-	1.00	417
Faba bean (Fb)	-	1.00 **a**	-	-	1.00	5904
Wheat (W)	-	-	1.00 **a**	-	1.00	5457
Mustard (M)	-	-	-	1.00**a**	1.00	13683
Fp+Fb	0.58**b**	0.21 **b**	-	-	0.79	1329
Fp+W	0.41 **bc**	-	0.13 **b**	-	0.54	1233
Fp+M	0.25 **c**	-	-	0.98**a**	1.23	12966

Kulumsa

Crop treatment	Fp	Fb	W	M	Total	Net benefit (birr ha^{-1})
Field pea (Fp)	1.00 **a**	-	-	-	1.00	2507
Faba bean (Fb)	-	1.00 **a**	-	-	1.00	10608
Wheat (W)	-	-	1.00 a	-	1.00	3912
Mustard (M)	-	-	-	1.00**a**	1.00	12938
Fp+Fb	0.76**b**	0.13 **b**	-	-	0.89	3109
Fp+W	0.95 **a**	-	0.23 b	-	1.18	2847
Fp+M	0.75 **b**	-	-	0.59 **b**	1.34	9431

* Market price of field pea @ Ethiopian birr 2.50/kg, Faba bean @ 2.00/kg, wheat 1.80/kg and mustard @ 3.50/kg; birr 8.50 = approximately US$ 1, April 2001.

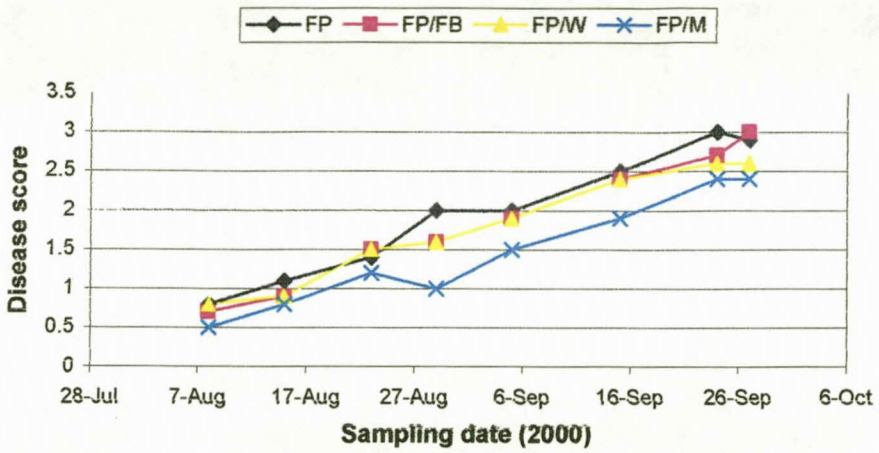

Figure 6.1 Ascochyta score (0-5 scale) in monoculture field pea (FP) and field pea/faba bean (FB), field pea wheat (W) and field pea mustard (M) mixed crops planted at Holetta, Ethiopia in 2000.

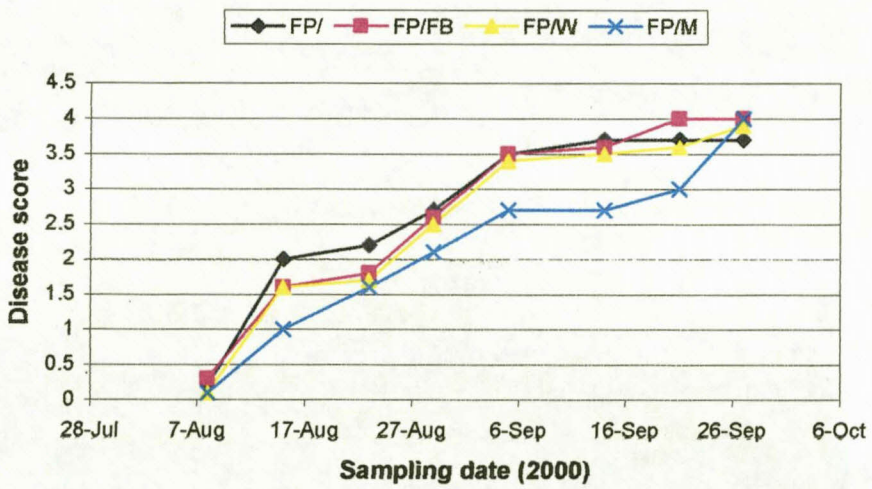

Figure 6.2 Ascochyta score (0-5 scale) in monoculture field pea (FP), field pea / faba bean (FB), field pea / wheat (W) and field pea / mustard (M) mixed crops planted at Denbi, Ethiopia in 2000.

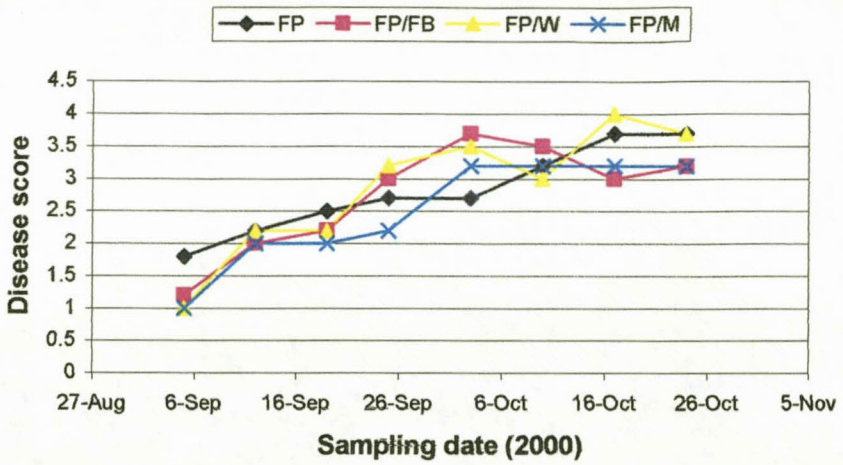

Figure 6.3 Ascochyta blight severity (0-5 scale) in monoculture field pea, field pea/faba bean, field pea/wheat and field pea/mustard mixed intercrop planted at Kulumsa, Ethiopia in 2000.

Appendix 1 Metreological data for the three field pea mixed cropping experimental sites, Holetta, Denbi and Kulumsa in Ethiopia over five years (1995-99) and in 2000.

Holetta

Month	Rainfall (mm)		Temperature (^{0}C)					
			1995-99			2000		
	1995-99	2000	Max	Min	Average	Max	Min	Average
January	41.7	0	23.1	4.1	13.6	23.3	0.3	11.8
February	32.9	0	24.7	3.7	14.2	24.5	0.6	12.6
March	105.0	12.5	24.5	7.4	15.9	25.7	3.5	14.6
April	72.0	123.8	24.1	8.0	16.0	23.8	7.3	15.6
May	56.2	52.0	24.3	7.5	15.9	23.5	6.9	15.2
June	129.3	89.9	22.8	7.5	15.2	21.7	6.5	14.1
July	259.2	187.1	19.6	8.6	14.1	20.2	7.7	13.9
August	245.3	260.6	21.5	8.6	15.0	18.9	7.6	13.2
September	100.5	120.7	20.9	7.0	13.9	20.1	6.6	13.3
October	41.42	9.5	21.9	5.1	13.5	21.5	4.6	13.1
November	5.16	38.9	22.4	2.1	12.2	22.7	2.3	12.5
December	8.81	30.4	22.7	1.2	12.0	22.4	2.5	12.4
Total	1097	925						
Mean			22.7	5.9	14.3	22.3	4.7	13.5

Appendix 1 continued...

Denbi

Month	Rainfall (mm)		Temperature (^{0}C)					
			1995-99			2000		
	1995-99	2000	Max	Min	Average	Max	Min	Average
January	19.3	0	26.7	8.1	15.8	27.1	6.1	16.6
February	10.2	0	28.5	7.1	17.8	28.0	6.8	17.4
March	43.1	24.7	24.2	12.2	19.6	29.8	10.2	20.0
April	68.4	28.3	27.8	11.6	19.7	29.4	13.2	21.3
May	36.6	55.8	29.4	12.0	20.7	28.3	12.5	20.4
June	108.1	59.8	27.9	12.4	21.1	27.7	11.3	19.5
July	239.1	219.4	24.2	13.1	18.7	25.1	13.1	19.1
August	239.0	196.2	24.5	13.3	18.9	23.9	13.2	18.5
September	97.2	142.8	26.2	11.8	19.0	25.1	12.4	18.7
October	71.3	24.8	26.3	9.0	17.7	25.7	8.2	16.9
November	16.2	49.2	25.5	6.5	16.0	25.9	7.1	16.5
December	0.46	3.8	25.6	4.9	15.3	25.5	7.0	16.2
Total	934	805						
Mean			26.4	10.2	18.4	26.8	10.4	18.4

Appendix 1 continued...

Kulumsa

Month	Rainfall (mm)		Temperature (^{0}C)					
			1995-99			2000		
	1995-99	2000	Max	Min	Average	Max	Min	Average
January	20.8	0	23.0	11.2	17.1	23.1	8.5	15.8
February	67.8	0	23.6	10.9	17.2	24.3	9.8	17.0
March	89.1	0.4	24.5	13.3	18.9	25.9	10.6	18.2
April	66.4	74.6	24.8	12.6	18.7	25.1	13.5	19.3
May	93.7	146.6	24.4	13.7	19.0	23.9	12.3	18.1
June	94.8	138.3	23.2	13.0	18.1	23.1	12.2	17.6
July	114.9	151.9	20.4	12.2	16.3	20.6	12.8	16.7
August	124.4	82.9	20.7	8.4	14.5	20.4	12.2	16.3
September	84.8	120.1	21.1	11.9	16.5	21.2	11.5	16.3
October	96.1	34.8	22.2	12.2	17.2	21.6	12.2	16.5
November	21.5	27.3	22.0	10.8	16.4	20.7	10.7	15.7
December	0	0	22.0	15.7	18.8	21.7	9.1	15.4
Total	874	776.9						
Mean			22.7	12.2	17.4	22.6	11.3	16.9

CHAPTER 7

Effect of sowing date and fertilizer on the severity of ascochyta blight and pea aphid incidence on three field pea varieties in Ethiopia.

Abstract

A field trial was carried out to investigate the effect of sowing date and fertilizer application (18 kg N and 46 kg P_2O_5 per hectare) on pea aphid infestation levels, ascochyta blight severity and crop yield. Field pea varieties used were Tegegnech and Adi (at Holetta), Mohanderfer and Markos (at Denbi and Kulumsa) and local farmers' varieties for the respective regions in Ethiopia. Response variables recorded included aphid count, ascochyta blight severity rating, pods per plant, seeds per pod, 100-seed weight and yield. Fertilizer or pea genotype did not significantly affect the pea aphid population densities, pods produced per plant, biomass and grain yields. However, ascochyta blight severity, number of days to 50% flowering and 90% maturity were significantly reduced by fertilizer application at the Denbi and Kulumsa sites. Neither early (mid-June) nor late (last week of June) sowing had any significant effect on pea aphid or blight severity at these two locations. Differences among genotypes were observed in terms of yield and other parameters. Even though fertilizer application at Denbi and Kulumsa had succeeded in reducing the severity of ascochyta blight in both early and late sowings, it did not result in a better yield due to the soil fertility status. The results suggested that fertilizer application significantly reduced the disease severity at Holetta, Denbi and Kulumsa but increased biomass and grain yield at the Holetta only compared with control. The interaction effects of variety, sowing date and fertilizer on disease severity, aphid per plant, pods per plant, grain yield and its components were not significant at all locations. From this investigation it can be concluded that

integrated management of ascochyta blight is possible with the integration of moderately resistant varieties and fertilization.

Key words: Field pea, varieties, sowing date, fertilizer, ascochyta blight, pea aphid.

Introduction

Field peas, *Pisum sativum* (L.) is one of the world's most important pulse crops. The total production of field pea has been estimated to be over 346,000 metric tones per annum in Africa (FAO 1999). However, the productivity per unit area is very low (0.6 t/ha) when compared with France (5.1 t/ha), UK (3.1 t/ha), Canada (2.1 t/ha) and China (1.8 t/ha). As is the case with other crops, small-scale and resource-poor farmers undertake field pea production in Ethiopia (Beyene, Nigatu & Woldemariam 1994). The low yields, among other things, are attributed to insect pests and diseases. According to Ali & Habtewold (1994) pea aphid is the most important pest of field pea in Ethiopia, particularly in the mid-altitude areas, whilst ascochyta blight is reported to be the most destructive disease in the country (IAR 1997, Gorfu 2000). The importance of these biotic constraints regarding field pea cultivation has also been reported in other parts of the world (Hagedorn 1985; Autrique, Starý, & Ntahimpera 1989; Bommarco 1992; Starý, Gerdoing, Norambuena & Remaudière 1993; Allard, Bill & Touraud 1993; Kraft & Kaiser 1993; Monti, Fruciante, & Romano 1993; Girousse & Bournoville 1994; Bhatnagar 1996). The control methods used against these pests are primarily chemical pesticide application. One of the main reasons farmers in Ethiopia are not readily using pesticides is the cost and unavailability at local markets.

Planting times and fertilizer application have been reported to have a pronounced influence on yield losses caused by aphids and ascochyta blight (Archer, Bynum, Onken &

Wendt 1995; Khattak, Khan, Shah, Zeb, & Iqbal 1996; Hammon, Paerson & Peairs 1996; Mittal 1997; Shafique, Anwar, Ashraf & Moula 1999; Bretag, Keane & Price 2000). Studies on the effect of nitrogen fertilizer on aphid population dynamics have been reported for several species of aphids (Omar, Haydar & Afifi 1993; Silva-Krott, Singh, Lali & Muniappan 1995; Singh, Yazdani, Veram & Singh 1995). However, studies in this regard on *Acyrthosiphon. pisum* or other aphids on field pea have not been documented.

In Ethiopia, the time of sowing and fertilizer application is determined by the importance that the farmer attaches to the crop. Normally, farmers sow their main crops, such as cereals (e.g. tef and wheat) first, after which subsidiary crops, such as field peas, are planted. With increasing costs of chemical fertilizers and farmers' inability to purchase them, little or no fertilizer is applied to legumes. This is because most farmers believe that legumes do not need fertilizer and they give priority to other crops such as cereals. Field pea planting is traditionally done from early June to mid-July depending on soil type. However, the majority of field pea fields in the main season (June to September) are planted between the second week of June and the third week of July (Beyene *et al.* 1994).

Cultural practices as a pest management tool is a neglected area of research that needs to be explored. Consequently, a study on the influence of two cultural practices (soil fertility and sowing date) on pea aphid infestation and disease severity and the application of these as a possible management strategy was conducted.

The objective of this study was to evaluate the effectiveness of fertilizer and sowing date on pea aphid population size and ascochyta blight infestation levels. From these data, practices could possibly be identified that could be implemented for pest and disease management.

Materials and Methods

The experiments were carried out at Holetta (3831E, 0903N; 2400 m a.s.l.), Kulumsa (3911E, 0803N; 2200 m a.s.l.) and Denbi (3859E, 0846N; 1900 m a.s.l.) Research Centers during the winter season of 2000. The mean monthly temperatures, monthly total rainfall and soil characteristics during the growing season of each site are presented in Table 6.1 and 6.2 (see Chapter 6).

Experimental units were established on fields where either winter wheat (at Kulumsa and Denbi) or potato (at Holetta) had been grown the previous year. Pre-sowing land preparation consisted of deep ploughing with either a disk or a moldboard plough, followed by cultivation with a disk harrow to break the clods. Prior to planting and after the first rains of the season the soil was again disked to control weeds and further refine the seedbed. Treatments were sowed around mid-June (early date) and 30 June (late date)), with or without fertilizer and three varieties were used. Field pea varieties used were Tegegnech and Adi (at Holetta), and Mohanderfer and Markos (at Denbi and Kulumsa). This selection was based on the suitability of the varieties to the respective environmental conditions. Local farmer varieties were used for the respective regions. Sowing dates were 16, 13, and 14 June at Holetta, Denbi and Kulumsa respectively for the first planting. The second planting was delayed at Denbi until soil moisture conditions permitted seed germination. There was enough rain at this site and the soil moisture was enough during the first planting. Field pea was hand seeded into previously opened furrows.

All treatment combinations were arranged factorially within a randomized complete block design and replicated 4 times. The plot size was 3 x 4 m with inter-row spacing of 20 cm. Each plot was separated from the adjacent plots by 1.5 m of bare ground. The

experimental plots (24 plots) were fertilized with diammonium phosphate (DAP) at the rate of 100 kg (containing 18 kg N and 46 kg P_2O_5) per hectare at sowing and mixed into the soil.

In July, emergence was assessed by counting the number of plants per square meter per plot. Population densities of pea aphid were assessed weekly for eight weeks, starting in mid-August. At each sampling day, counts were done on 10 individual plants chosen randomly per plot. The numbers of aphid mummies were recorded on these ten plants as well, while the number of predators was assessed on a plot basis.

Severity of ascochyta blight was recorded visually every week per plot on a severity scale of 0-5 (Roger, Tivoli & Huber 1999). Meteorological records of the respective locations, including rainfall and temperature, were obtained from the stations. Measurements were made of plant stands at emergence, 50% flowering, plant height, days to maturity, pods per plant, seeds per pod, 100-seed weight, total biological (= bio-mass) and grain yields.

Statistical analysis was conducted using the MSTAT-C (1990) software package. A three-way factorial analysis of variance (Steel & Torrie 1980) was performed to compare varieties, sowing date and fertilization, with mean separation using the Fisher least significance difference (LSD) test. The MSTAT-C subprogram FACTOR was used to perform the factorial analysis. Analysis was performed on data collected at each location with sowing date and fertilizer rate of the three varieties as independent variables.

Results

Analysis of variance on stand count at emergence was initially performed separately for each location, but the test for homogeneity of variance showed non-significant χ^2 values at Denbi and Kulumsa. Therefore, the data for all variables for the two locations were pooled and analyzed and subsequent interpretations were based on the combined analysis.

The analysis of variance for pea aphid population density and ascochyta blight severity at Holetta are summarized in Table 7.1. The probability values for the partial ANOVA (Table 7.1) indicated that fertilizer application significantly ($P<0.001$) influenced the density of pea aphid population and ascochyta blight severity at Holetta. Fertilizer, sowing date and field pea variety interactions were nonsignificant, indicating that the three factors had little or no effect on each other. There was no difference in disease reactions among the three varieties used in the trail at Holetta. To the contrary, the three varieties showed significant differences ($p<0.01$) in pea aphid population densities, with the lowest incidence on cultivar (cv) Adi. The fertilizer - sowing date interaction was also not significant for either pea aphids or ascochyta blight. The sowing date was not effective in reducing pea aphid population sizes and no difference in the disease score was detected, probably because the time gap was not wide enough. The lack of significant differences can probably be explained by the dates which fall within the range of recommended sowing dates for field pea at these sites.

Pea aphid populations started with low numbers and increased gradually until they reached a peak of abundance in the first week of September. This dramatic increase occurred during flowering and early pod setting stage of the crop. Pea aphid population density was not significantly affected by either fertilizer application or by sowing dates, both at Denbi and Kulumsa (Table 7.2). However, fertilizer - sowing date interactions was significant for pea aphid infection ($p<0.05$). Significant differences were found among fertilizer application ($p<0.001$), varieties ($p<0.01$) and location ($p<0.001$) and an interaction between the three existed ($p<0.05$) for ascochyta blight (Table 7.2). Fertilizer applications also substantially reduced the ascochyta blight severity at Denbi and Kulumsa on all varieties tested (Table

7.3), with an overall mean rating scale of 2.8 (range 2.6-3.2) for unfertilized plots. The comparative figure for fertilized plots was 2.0 (range 1.8-2.4). The three varieties did show differences in disease reactions at Denbi and Kulumsa (refer to Appendix 2b). At Denbi, cv Markos was moderately resistant to ascochyta blight with a mean score of 2.2. At Kulumsa, cv Markos (2.5) and the farmers' variety (2.4) were significantly different from cv Mohanderfer with a mean score of 2.8. The higher mean score recorded was on cv Mohanderfer on the unfertilized plots, indicating that this variety was more susceptible to the disease than the two varieties at either of the two locations. Furthermore, Kulumsa had the higher mean score (2.5), when compared with that of Denbi (2.3) (refer to Appendix 2a). In terms of sowing dates, ascochyta blight severity showed no significant differences between dates.

The pea aphid population density and ascochyta blight severity varied widely over the three locations. The mean total numbers of aphids/plant and ascochyta blight severity at Holetta were comparatively low compared with Denbi and Kulumsa (Figures 1 and 2).

Predator (Coccinellidae beetles and Syrphidae flies) populations were of negligible importance in terms of biological control. Although predators were present in association with aphids, their numbers oscillated during the season and they did not suppress the increase in *A. pisum* numbers in late August and September. Parasitism of pea aphids by *Aphidius* spp. (Braconidae) was high and similar in all treatments. Parasitoid abundance was dependent on pea aphid density and parasitoids failed to reduce pea aphid numbers before field pea damage exceeded the economic injury level. Parasitoid activity was first observed in late August or early September, coinciding with the maximum population sizes of pea aphids. The percentage of pea aphids' parasitised varied between locations. However, the

extent of parasitism ranged between 4.5% and 22.9%, with an average of 10.2% at Holetta, 6.9% (range 2.7 – 18.1%) at Denbi and 4.5% (range 1.5 – 7.9%) at Kulumsa. The parasitoid activity continued up to early October.

Results of the ANOVA for yield and components are summarized in Tables 7.4 and 7.5. There was also a significant effect resulting from fertilizer application on most of the attributes under study at Holetta. The biomass and grain yields, pods per plant and 100-seed weight increased significantly in the fertilized plots versus the non-fertilized ones (Table 7.4). The use of fertilizer significantly increased pods per plant, biomass and grain yields by 12, 21 and 28% respectively over the control at Holetta. This increase was primarily due to balanced nutrients in the soil under the fertilized condition. The fertilizer supplied nutrients to the crop thereby increasing the yield, with apparent nutrient deficiencies according to the data from soil analysis (see Chapter 6, Table 6.1). It would therefore appear that fertilization with N and P was crucial for harvesting optimum crop yield at Holetta.

The analysis of variance with regard to number of days to flowering and maturity, plant height, pods per plant, 100-seed weight, biomass and grain yield revealed that there were significant differences between varieties for all these parameters, except for the number of seeds per pod and biomass yield (Table 7.4). Variety - fertilizer interaction was significant only in days to flowering. However, there were significant differences ($P< 0.01$) between sowing dates and grain yield, i.e. as the sowing date was delayed, the grain yield was significantly reduced.

The biomass yield of the crop sown late provided a significantly higher yield (6417 kg/ha), compared with the crop that was sown early (5625 kg/ha). This suggested that sowing performed in the last week of June should be the preferred date for Holetta areas.

Plots fertilized with DAP also significantly yielded higher biomass (6750 kg/ha), when compared to unfertilized ones (5333 kg/ha).

Neither the fertilizer, nor the sowing date had any statistically significant effect on the number of pods per plant, seeds per pod, biomass and seed yields for the three varieties of field pea used in the study at Denbi and Kulumsa (Table 7.5). Fertilizer application did, however, induce earlier flowering and maturity of field pea plants at Denbi and Kulumsa.

At Denbi, the mean number of pods per plant was similar among the three varieties. At Kulumsa, however, the number of pods produced per plant in the case of the two improved varieties was significantly higher than that of farmers' variety (refer to Appendix 2d). Generally, the mean number of pods per plant recorded at Kulumsa was significantly higher (11.4) to that of Denbi (6.7) (Table 7.6). This obviously attributed to the lower grain yields obtained at Denbi (refer to Appendix 2c). The number of seeds produced per pod was the highest for cv Markos, than with the other two varieties (refer Appendix 2e). The biomass yield of cv Markos was also higher than the other varieties in both fertilized and unfertilized plots (Table 7.7). The mean biomass yield for cv Markos across the two locations was significantly higher than the other varieties when sown in the first week of July (refer Appendix 2f and 2g). The location - fertilizer - sowing date - variety interactions were significant for biomass yield, showing that the effect of one factor was influenced by the other three (Tables 7.8).

The variety by fertilizer interactions was significant ($P< 0.05$) for seed yield, indicating that varieties responded differently to fertilizer application (refer to Appendix 2h). At Denbi unfertilized plots (452 kg/ha) produced significantly more yield than fertilized plots (273 kg/ha) (refer to Appendix 2i). At Kulumsa, the farmers' variety performed the worst,

providing a significantly lower yield than the two improved varieties (Table 7.9). The mean seed yield recorded at Kulumsa (918 kg/ha) was more than double when compared with that of Denbi (362 kg/ha) (Table 7.9). The Markos cultivar is consistently the best performer in terms of overall yields and its reaction to ascochyta blight severity. The seed size is larger than that of the other varieties, while the number of pods per plant approaches levels found in Mohanderfer and the local variety.

Discussion

The present study indicates that fertilizer application and pea aphid population size and ascochyta blight severity on field pea is location specific. Ascochyta blight infection and pea aphid population density were significantly reduced in fertilized plots when compared with unfertilized plots at Holetta, while it was only ascochyta blight that showed a significant difference at Denbi and Kulumsa. Result also indicate that fertilizer application was found to be the most important factor that affects pods per plant, 100-seed weight biomass and grain yields at Holetta. It also has a pronounced effect on ascochyta severity, flowering and maturity dates at Denbi and Kulumsa.

Pea aphid pressure that was usually lower at Holetta than at the other two sites is believed to be attributed to the low temperature and high rainfall. Heavy rainfall has been shown to cause aphid mortality (Watson & Carter 1983; Mann, Tatchell, Dupuch, Harrington, Clark & McCartney 1995). According to Ali (1999), pea aphid populations tend to crash at higher rainfall and low temperature despite the availability of susceptible pea plants. Lack of significant interaction between fertilizer treatment and aphid infestation suggested that aphids affected yields similarly for all the fertilizer treatment studies. Riedell & Kieckhefer (1993) reported similar results with wheat and the Russian wheat aphid.

A sharp increase in ascochyta blight infection and a decrease in yield in the unfertilized plots at Holetta indicated the importance of fertilizer in controlling this disease. However, the reduction in ascochyta blight severity observed in fertilized plots did not improve grain yields at Denbi and Kulumsa. At Denbi specifically, a yield reduction of about 40% was noted when fertilizer was applied. A possible reason for this phenomenon could be that fertilizer caused lodging that resulted in yield reduction. Data from soil analyses supported this assumption (see Chapter 6). A balanced fertilizer to reduce ascochyta blight damage and to increase biomass and grain yields under Holetta conditions is therefore recommended, provided the application is economically justifiable.

Fertilized plants probably were more vigorous due to the availability of more nutrients, and therefore offered less favorable conditions for disease infection and development. Furthermore, infected plants in fertilized soils managed the damage factor by rapidly growing through the critical injured stage and as a result ascochyta blight infection had less effect on grain yield.

The nutritional status of a plant can have a significant impact on disease susceptibility and insect attack levels. Haltrich, Janos, Jozsef & Laszlo (2000) found out that nitrogen (N) resulted in reduced aphid infestation on apple cultivars. To the contrary, Ali & Ahmed (1996) confirmed that an increase in N fertilizer significantly increased aphid infestation on wheat. Atiyeh, Aslam & Baalbaki (1996) have reported similar findings on sweet corn. In another study, Khattak *et al.* (1996) showed that the application of N fertilizer alone increased aphid incidence, while N and phosphorous (P) in combination suppressed aphid attack and tripled rapeseed yield, when compared to the control. These observations are in contrast with a report by Archer *et al.* (1995) which, stated that aphid densities are not

different amongst different rates of N fertilizer application. This disparity could be due to differences in methodology, crop and aphid species used in the study. More recently it has been shown that the amount of soluble N that affects aphid growth most significantly differs between aphid species. In this context, the preferred amount of amino acid is 3% for *Myzus persicae*, whilst *A. pisum* requires a level of exactly 4.3% (Haltrich *et al.* 2000). Phosphorous deficiency has been found to alter insect-plant interactions in that it is associated with the structural components of plants and affects the metabolism of sugar phosphates, nucleic acids, nucleotides, coenzymes and phospholipids (Clark 1982). The concentration of phosphorous may also alter several biochemical processes in plants and thus affect specific nutritional requirements of insects that feed on such plants.

On the other hand, neither early nor late sowing affected infection levels at any of the locations. A wider range of sowing dates than was used in this study might help to clarify the effects of sowing dates on ascochyta blight and pea aphid severity. Several workers have reported the relative advantage of early sowing over late sowing of different crops to minimize pest damage. Hammon *et al.* (1996); Shafique *et al.* (1999) and Karungi, Adipala, Kyamanywa, Ogenga–Latigo, Oyobo & Jackai (2000), for example, all demonstrated that early sowing helped to manage aphids in different crop species. According to Karungi *et al.* (2000) the reduction in aphid populations with early planting is attributed to the lower aphid population density early in the season, which only builds up as the season progresses. These authors also suggested that heavy rainfall early in the season could kill aphids. Early sowing was also found to be most effective in reducing disease infection, including ascochyta blight, in lentils (Tripathi, Chaube & Singh 1986; Eser, Aydin & Adak 1991 and Mittal 1997). All

in all therefore, the effect of sowing date on pest incidence on any crop is the result of different factors such as climate, variety, cropping system and pest species, amongst others.

Present results also indicate that pea variety Markos is moderately resistant to ascochyta blight and delivers higher yields, when compared with Mohanderfer and farmers' varieties. The fact that genotype x fertilizer x sowing date and location was significant for ascochyta blight severity indicated that as far as the disease is concerned, the effect of one factor was influenced by the other two factors. For pea aphids and yield, on the other hand, the three factors had little or no effect on one another. It can be concluded that integrated management of ascochyta is possible with moderately resistant cultivars and fertilization. However, neither early nor late sowing affected ascochyta blight nor pea aphid infection levels at any of the locations.

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Table 7.1. Probability values for the partial ANOVA on the effect of fertilizer to ascochyta blight severity and pea aphid population size on three field pea varieties grown at Holetta (Ethiopia), planted early and late in the growing season.

Source	DF	Disease Score	Aphid/plant
Replication (R)	3	NS	NS
Fertilizer (F)	1	0.00001	0.0007
Sowing date (SD)	1	0.0829	NS
F x SD	1	NS	NS
Variety (V)	2	NS	0.0023
F x V	2	NS	NS
SD x V	2	NS	NS
F x SD x V	2	NS	NS
Error	33		
CV (%)		9.13	32.7

NS = non-significant; DF = degree of freedom

Table 7.2. Probability values for the partial ANOVA on the effect of fertilizer to ascochyta blight severity and pea aphid population size on three field pea varieties grown at two locations (Denbi and Kulumsa, Ethiopia). Field peas planted early and late in the growing season.

Source	DF	Disease Score	Aphid/plant
Replication (R)	3	NS	0.0184
Fertilizer (F)	1	0.00001	NS
Sowing date (SD)	1	0.0740	NS
F x SD	1	NS	0.01811
Variety (V)	2	0.0073	NS
F x V	2	NS	0.0647
SD x V	2	NS	NS
F x SD x V	2	NS	NS
Location (L)	1	0.00001	0.00001
F x L	1	0.0330	NS
SD x L	1	NS	NS
F x SD x L	1	NS	NS
G x L	2	0.0446	NS
F x V x L	2	0.0365	0.0697
SD x V x L	2	NS	NS
F x SD x V x L	2	0.0142	NS
Error	69		
CV(%)		12.7	43.1

DF = degree of freedom; NS = non-significant

Table 7.3. Disease score (0-5 scale) of three field pea varieties grown at two locations (Denbi and Kulumsa, Ethiopia) and two sowing dates.

Location	Variety	Fertilizer*		Mean
		Without	With	
Denbi	Mohanderfer	2.8**bc**	1.8**g**	2.3
	Markos	2.6**cd**	1.9**g**	2.2
	Local	2.8**bc**	1.8**g**	2.3
Kulumsa	Mohanderfer	3.2**a**	2.4**de**	2.8
	Markos	2.9**ab**	2.0**fg**	2.4
	Local	2.6**bcd**	2.2**ef**	2.4
Mean (overall)		2.8	2.0	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD; data are combined over sowing date.

Table 7.4. Probability values for the partial ANOVA of the effect of fertilizer on agronomic parameters and yield for three field pea varieties grown at Holetta (Ethiopia), planted early and late in the growing season.

Source	DF	P/P	S/P	H	FD	MD	BY	SY	100-SW
Replication (R)	3	NS	0.0393	NS	NS	NS	NS	NS	NS
Fertilizer (F)	1	0.0323	NS	NS	0.00001	NS	0.0021	0.00001	0.0086
Sowing date (SD)	1	0.0770	NS	0.0064	NS	NS	NS	0.0021	NS
F x ST	1	NS	NS	NS	0.0003	NS	0.0499	NS	NS
V	2	0.0181	NS	0.0400	0.0003	0.0216	NS	0.0014	0.00001
F x V	2	NS	NS	NS	0.00001	NS	NS	NS	NS
SD x V	2	NS	NS	NS	NS	NS	NS	NS	NS
F x SD x V	2	NS	NS	NS	NS	NS	NS	NS	NS
Error	33								
CV(%)		25.8	10.9	11.7	1.8	5.7	24.2	23.5	7.1

DF = degree of freedom; P/P = pod/plant; S/P = seed/pod; H = plant height; FD = 50% flowering date; MD = 90% maturity date; BY = biomass yield; SY = seed yield; 100-SW = 100-seed weight.

Table 7.5. Probability values for the partial ANOVA of the effect of fertilizer on agronomic parameters and yield for three field pea varieties grown at Denbi and Kulumsa, planted early and late in the growing season in Ethiopia.

Source	DF	P/P	S/P	H	FD	MD	BY	SY	100-SW
Replication (R)	3	NS	NS	NS	NS	NS	NS	0.0841	NS
Fertilizer (F)	1	NS	NS	NS	0.0199	0.00001	NS	NS	NS
Sowing date SD)	1	NS	NS	NS	NS	NS	0.0146	NS	NS
F x SD	1	NS	NS	NS	NS	NS	NS	NS	NS
V	2	0.0006	0.0008	0.00001	0.0003	0.0118	0.0055	0.00001	0.00001
F x V	2	NS	NS	NS	NS	NS	NS	0.0423	NS
SD x V	2	NS	NS	NS	NS	NS	0.0007	NS	NS
F x SD x V	2	NS	NS	NS	NS	NS	0.0001	NS	0.0078
Location (L)	1	0.00001	0.00001	NS	0.00001	0.00001	0.00001	0.00001	0.0001
F x L	1	NS	NS	0.0011	NS	0.00001	NS	0.0005	NS
SD x L	1	NS	0.0356	NS	NS	NS	0.0026	NS	NS
F x SD x L	1	0.0205	NS	NS	NS	NS	NS	NS	NS
V x L	2	NS	0.0066	NS	0.0042	NS	NS	0.00001	NS
F x V x L	2	NS	NS	NS	NS	NS	NS	NS	NS
SD x V x L	2	NS	NS	0.0550	NS	NS	0.0014	NS	NS
F x SD x V x L	2	NS	NS	NS	NS	NS	0.0003	NS	NS
Error	69								
CV(%)		22.7	11.6	7.3	5.2	3.0	19.7	27.1	10.1

NS = non-significant

DF = degree of freedom; P/P = pod/plant; S/P = seed/pod; H = plant height; FD = 50% flowering date; MD = 90% maturity date; BY = biomass yield; SY = seed yield; 100-SW = 100-seed weight.

Table 7.6. Main effects for pods per plant of three field pea varieties grown at Denbi and Kulumsa in Ethiopia.

Fertilizer	Sowing date	Location *		Mean
		Denbi	Kulumsa	
With	Early	5.7d	11.3ab	8.5
	Late	5.4d	12.3a	8.8
Without	Early	7.5c	12.0a	9.7
	Late	8.3c	10.1b	9.2
Mean		6.7	11.4	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over varieties.

Table 7.7. Effects of sowing date and fertilizer on biomass yield (Kg/ha) of three field pea varieties grown at two locations in Ethiopia.

Fertilizer	Variety	Sowing date*		Mean
		Early	Late	
With	Mohanderfer	4250bc	3750cd	4000
	Markos	5250a	4833ab	5041
	Local	2833e	5167a	4002
Without	Mohanderfer	3417de	4917ab	4167
	Markos	4583abc	4083bcd	4333
	Local	4000cd	4250bcd	4125
Mean (overall)		4055	4500	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over locations.

Table 7.8. Main interaction effect of location x variety x sowing date x fertilizer application on biomass yield (kg/ha) of three field pea varieties at two locations in Ethiopia.

Location	Variety	Sowing early*		Sowing late	
		Fertilized	Unfertilized	Fertilized	Unfertilized
Denbi	Mohanderfer	3083gh	2583h	2667h	2667h
	Markos	3500gh	3000h	3167h	3083h
	Local	2417h	2333h	2333h	2417h
Kulumsa	Mohanderfer	5417cde	4250fg	4917ef	7083ab
	Markos	7000ab	6083bcd	6583bc	5000def
	Local	3250gh	5583cde	7917a	6083bcde

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date.

Table 7.9. Main interaction effect of variety x location on grain yield (kg/ha) of three field pea varieties with and without fertilizer at two locations in Ethiopia.

Variety	Location*		Variety mean
	Denbi	Kulumsa	
Mohanderfer	370d	933b	651
Markos	349d	1259a	804
Local	368d	563c	465
Location mean	362	918	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date.

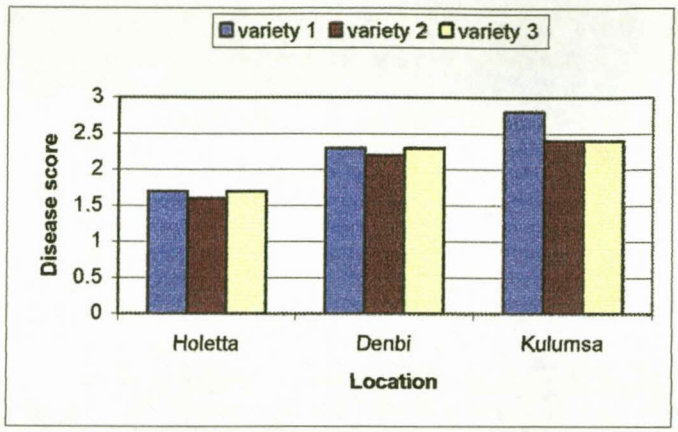

Figure 7.1 Ascochyta blight severity (0-5 scoring scale) for the three field pea varieties recorded at the three experimental sites in Ethiopia. Mean scores of eight scoring dates.

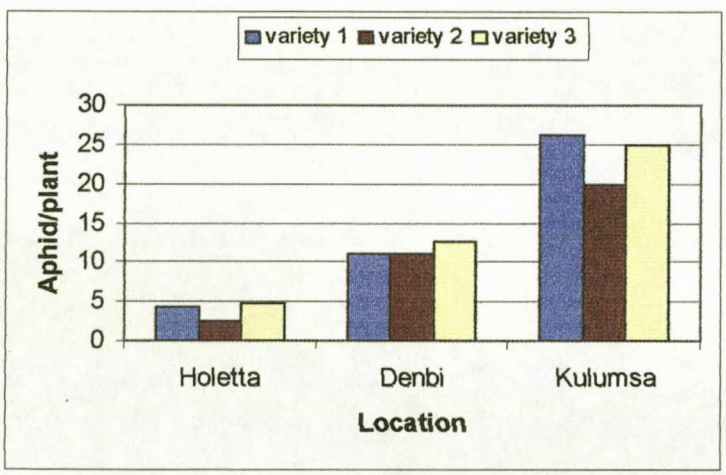

Figure 7.2 The pea aphid population density observed at the three experimental sites in Ethiopia during the 2000 growing season. Mean of eight counts per season.

Appendix 2(a) Disease score (0-5 scale) of field pea varieties grown at two locations in Ethiopia with and without fertilizer applications.

Fertilizer	Location*		Fertilizer mean
	Denbi	Kulumsa	
With	1.8c	2.2b	2.8
Without	2.7a	2.9a	2.0
Location mean	2.3	2.5	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are pooled over sowing date and varieties.

Appendix 2(b) Disease score (0-5 scale) of field pea varieties grown at two locations in Ethiopia, with and without fertilizer applications and two sowing dates.

Variety	Location*		Variety* mean
	Denbi	Kulumsa	
Mohanderfer	2.3b	2.8a	2.5
Markos	2.2a	2.5b	2.3
Local	2.3b	2.4b	2.3
Location mean	2.3	2.6	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over three varieties.

Appendix 2(c) Effect of fertilizer on pods per plant of three field pea varieties grown during the two sowing dates at Denbi and Kulumsa in Ethiopia.

Fertilizer	Location*		Fertilizer mean
	Denbi	Kulumsa	
With	5.6c	11.8a	8.7
Without	7.9b	11.1a	9.5
Location mean	6.7	11.4	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date.

Appendix 2(d) Effect of fertilizer on the number of pods per plant and field pea varieties grown during two sowing dates at Denbi and Kulumsa in Ethiopia.

Variety	Location*		Variety mean
	Denbi	Kulumsa	
Mohanderfer	7.0c	12.1a	9.5
Markos	6.2c	13.3a	9.7
Local	6.9c	8.8b	7.8
Location mean	6.7	11.4	

*Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date.

Appendix 2(e) Main effects of fertilizer on seeds per pod of three field pea varieties grown at two locations in Ethiopia.

Variety	Location*		Variety mean
	Denbi	Kulumsa	
Mohanderfer	3.4c	3.9b	3.65
Markos	3.7b	4.5a	4.10
Local	4.0b	4.0b	4.00
Location mean	3.7	4.1	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date and fertilizer treatment.

Appendix 2(f) Main interaction effect of variety x sowing date on biomass yield (kg/ha) of three field pea varieties at two locations in Ethiopia.

Variety	Sowing date*		Variety mean
	Early	Late	
Mohanderfer	3833bc	4333ab	4083
Markos	4917a	4500a	4708
Local	3417c	4667a	4042
Sowing date mean	4056	4500	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test.

Appendix 2(g) Main interaction effect of variety x sowing date on biomass yield (kg/ha) of three field pea varieties grown early and late at two locations in Ethiopia.

Location	Sowing date*		Location mean
	Early	Late	
Denbi	2833c	5333c	2791
Kulumsa	2750b	6250a	5791
Sowing date mean	4083	4500	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date.

Appendix 2 (h) Main interaction effect of variety x fertilizer on grain yield (kg/ha) of three field pea varieties at two locations in Ethiopia.

Variety	Fertilizer*		Variety mean
	With	Without	
Mohanderfer	640c	663bc	651
Markos	828a	781ab	804
Local	380b	552c	466
Location mean	616	665	

* Means within column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test; data are combined over sowing date.

Appendix 2(i) Main interaction effect of fertilizer x location on grain yield (kg/ha) of three field pea varieties at two locations in Ethiopia.

Fertilizer	Location*		Fertilizer mean
	Denbi	Kulumsa	
With	273c	958a	615
Without	452b	878a	665
Location mean	362	918	

* Means within a column followed by the same letters are not significantly different from each other at the 5% probability level using LSD test

CHAPTER 8

Effect of neem insecticide formulations on *Acyrthosiphon pisum* (Harris) development and reproduction on field pea in Ethiopia

Abstract

The effects of a neem seed extract aqueous solution and a commercial neem product, Multineem®, on the metamorphosis, longevity and fecundity of pea aphid, *Acyrthosiphon pisum*, were determined in greenhouse studies. Pea aphid adults and young nymphs were exposed to treated plants and topical spray of the products. Pirimor 50% (wettable powder) aphicide was used as a comparison to neem treatments. The products significantly reduced population increase of *A. pisum* in a concentration-dependent manner. At a concentration equivalent to 100 mg liter^{-1} of azadirachtin (Multineem®), pea aphid population increase was 37% lower than in the control, whilst with the 10% aqueous seed extract (SE) this figure was 62%. Neem formulations significantly reduced the number of molts, longevity and fecundity of pea aphids that had been reared on treated field pea plants. The longevity and fecundity of young adults exposed to neem were also reduced, but were not as drastic as was the case in immatures. Due to especially SE, acute toxicity leading to immediate mortality of young nymphs was caused, whilst chronic effects resulting in a smaller progeny and reduced survival capabilities were also recorded. The 12 day LC_{50} for individuals exposed to SE from birth was 49.29 mg azadirachtin liter^{-1}, while the 12 day LC_{50} for adults was 440.94 mg liter^{-1}. The LC_{50} value for adults topically sprayed was 60.20 mg liter^{-} after 20 days

Key words: *Acyrthosiphon pisum*, field pea, azadirachtin, neem, development, toxicity.

Introduction

Among the key pests of field pea, the pea aphid (*Acyrthosiphon pisum*) is considered to be the most important agent in reducing yields and lowering the performance of the crop in Ethiopia. To increase agricultural production, it is necessary to keep insect pest populations below an acceptable threshold determined by the relation between economic losses caused by pests and the costs of treatments for their control. Chemical control of the pea aphid has been the only option for many growers and will remain so for some time. Synthetic pesticides used during the past 40 years often gave economical and effective pest control, which significantly enhanced the production of agricultural commodities. However, the indiscriminate use of some products caused environmental contamination, adverse effects on nontarget organisms and led to a build-up of resistance in pest populations. In this scenario, use of plant-derived pesticides should assure a selective activity against target pests and a shorter persistence in ecosystems (Stark & Walter 1995). Furthermore, pesticides of plant origin are cheap, readily available and cost effective in developing countries where synthetic pesticides are scarce and expensive for resource-poor farmers. Azadirachtin is one of the most important plant-derived compounds used in insect pest control, since it has very low mammalian toxicity and is relatively safe to beneficial insects (Schumutterer 1990, Spollen & Isman 1996, Markandeya & Dirakan 1999, Gahukar 2000).

Derivatives of neem, *Azadirachtin indica* A. Juss. (Meliaceae) have been used traditionally by small-scale farmers in Asia and Africa to protect crops from pests, both in the field and in storage. The neem plant is also widespread in many countries in Africa. Butterworth & Morgan (1968) first isolated this teteranortriterpenoid from the seeds of the neem tree. Neem compounds act as insect repellents and growth and reproduction inhibitors, among others, on more than 200 species of arthropods (Saxena 1989, Koul, Isman & Ketkar

1990). Of these bio-activities, disruption of development (molting failure) is considered to be the most economically significant effect (Wood 1990). As in the case of other botanical insecticides, neem extracts have a very low persistence in the environment because of this high sensitivity to UV light (Barnby, Yamasaki & Klocke 1989). For this reason, application of azadirachtin sprays should be frequent to be effective (Schmutterer 1990).

The susceptibility of aphids to neem is variable–and is probably dependant on the aphid species, the stage of nymphal instars and the host plant (Lowery & Isman 1994). Because of its selectivity and relatively minor impact on beneficial arthropods, neem-based insecticides appear to be highly suitable for use in IPM programs (Schmutterer 1990).

The objective here was to assess the possibility of including neem-based insecticides in pea aphid management under study in different parts of the world. The studies reported here were conducted by setting up four different experiments. The effect of neem formulations was compared on *A. pisum* when individuals were exposed to treated plants and when they were exposed by topical application to determine whether one method of exposure was more effective than another in terms of activity.

Materials and Methods

General. Pea aphids used in the four experiments that were set up were the offspring of small groups of apterous parthenogenic females collected from field pea plants at Holetta Agricultural Research Center, Ethiopia in June 2000. The colony was maintained continuously on Mohanderfer field pea in the same environment as the one in which the study was conducted.

The insecticide evaluated in this study was Multineem® 0.03% EC that is a natural insecticide derived from the neem tree that contains 3g/l azadirachtin, it's primary active

ingredient. Multineem® was obtained from International Center for Agricultural Research on the Dry Areas (Aleppo, Syria) and consisted of 0.03 % active ingredient of azadirachtin in a liquid formulation.

For the water extract neem seed extract was prepared from ripe neem fruits that were collected from neem trees at Melka Werer (750 m a.s.l.), Ethiopia. Seeds from mature dehisced fruits of neem were air-dried for one week. The seed coats were then split to remove the cotyledons. The seeds were then ground in a mortar and pestle to obtain 20g of paste. A cold water extract was prepared by placing the paste in cheesecloth suspended in a beaker containing 200 ml and 400 ml of distilled water over night. The resultant neem water extracts were 10 % and 5 % formulations, respectively. After more or less 12 hours the seed material was removed and squeezed out, after which the solution was stirred and sprayed.

Experiment 1. Effect of Multineem® and neem seed extracts on population increase.

Five seeds of the susceptible pea cv. Mohanderefer were planted into each of 100 pots (20 cm diameter circular pots) in the greenhouse at 25.2^{0}C (day) and 15.2^{0}C (night) and relative humidity (70-94%) under natural photoperiod. When the plants were two weeks old, they were sprayed with water and/or one of the seven concentrations of the neem formulation Multineem® (i.e. 0, 10, 20, 40, 60, 80 and 100 mg azadirachtin liter^{-1}) (Stark & Wennergren 1995). Five percent and 10% of the seed extract were used for comparison with commercial neem formulation and Pirimicab 50% WP (0.5% concentration) as a standard check. Five milliliters of insecticide solution (just enough to wet the upper and under surfaces of all leaves completely) was applied to each group of plants using a small hand-held sprayer. Ten aphids that were confined to potted plants treated with distilled water alone were included to assess natural mortality. After the plants were dried (for about 30 minutes), ten young adult

aphids (approximately 24 hours old) were placed on the soil at the base of the plants. Aphids and the plants were contained by clear plastic cylindrical cages (60-cm tall and 15-cm diameter) with a cloth top and six cloth-covered ventilation holes on the side. The pots were arranged in a completely randomized design on the greenhouse bench. Seven days after infestation, all the aphids were removed and counted. The experiment was replicated ten times (10 pots per treatment). Data for Pirimicarb were excluded from the analysis in all experiments since 100% mortality was recorded within a day after treatment.

Experiment 2. Effect of neem on longevity and reproduction of newly born pea aphids.

To determine the toxicological effects of neem formulation and seed extract on pea aphid, pea plants were treated as described in Experiment 1, with the following modifications. After plants are sprayed and dried, four young apterous adult female aphids were placed on each potted plant. Twenty-four hours after introduction of adults, all aphids were removed except for one first-instar in each pot. This ensured that newly born aphids were exposed to the treatments at birth. Mortality, number of molts and the number of offspring produced were recorded daily throughout the life span of the pea aphids. Aphids on treated plants were considered dead if they did not move their legs or antennae when prodded with a soft camelhair brush. Molting was determined by counting and removing cast skins daily. The life table was constructed using the parameters described above. Twenty aphids were used per concentration.

Experiment 3. An identical study to that described above was conducted with young adult aphids (24 hours old), instead of the newly born nymphs. Mortality and reproduction were recorded daily for adults throughout their life span.

Experiment 4. Topical toxicity

The same concentrations of azadirachtin (0, 10, 20, 40, 60, 80, and 100 mg^{-1}) were used in topical toxicity studies as described above. Batches of 10 adult pea aphids (24 hours old mean weight 3.14 mg) were treated topically by applying 0.59 µl of the neem insecticide formulation in distilled water to the dorsum of the thorax with a Hamilton syringe. After air-drying for 30 minutes, the adults were transferred onto a clean untreated pea plant and contained in pots as described above. Mortality and the number of offspring produced were recorded daily throughout their life span. Each concentration of the treatments had 20 aphids per concentration.

Data analysis. Mean total reproduction, mean numbers of molts and mean survival of individuals exposed as newly born nymphs and adults were analyzed by one-way analysis of variance (ANOVA) and means were separated by Duncan's Multiple Range Test at $P \leq 0.05$ (MSTAT-C 1990). Concentration-mortality regressions were estimated by Probit analysis (Finney 1971) using the SPSS (1994) Probit procedure from data generated in the longevity studies and the topical toxicity study.

Results

Experiment 1. Population increases. Pirimor (0.5%) used as standard check gave 100% mortality from 0 to 1 day after treatment; hence it was excluded from the analysis in all experiments. The final number of aphid populations exposed to the commercial neem product Multineem® and the aqueous seed extract (SE) are shown in Table 8.1. A significantly ($P < 0.05$) reduced rate of increase of *A. pisum* populations, comprised of nymphs and adults, was recorded at 10 days after treatment on plants applied with ≥ 20 ppm (Multineem®) and SE than was observed on control plants (Table 8.1).

Population number at the highest concentration of Multineem® (100 ppm) tested was 63 % of that of the control, whereas the 10 % SE was only 38 %. The SE proved to be very toxic compared with the Multineem®. In general, the two neem formulations significantly reduced aphid numbers in a dose-dependent manner.

Experiment 2. Pea aphids exposed to treated plants from birth. It is evident from Table 8.2 that the neem formulations Multineem® and SE had a profound effect on the biology of *A. pisum*. *A. pisum* nymphs were highly susceptible to SE compared to Multineem®. The exposure of the nymphs to Multineem® treated field pea plants resulted in significant concentration-dependent reductions in the number of molts (Table 8.2). The molting process was completely disrupted at the two levels of SE, averaging less than one molt. Number of molts for *A. pisum* from different treatments was influenced by treatment and rates and differences between the high rates ($\geq$ 40 ppm) and low rates were significant ($p<0.05$). The rates up to 20 ppm had little effect on the number of molts. Symptoms in immature *A. pisum* exposed to higher concentrations of Multineem® and SE included a failure to molt and grow and a change in color from green to yellow and eventually brown.

Survival of *A. pisum* nymphs on plants treated with Multineem® was significantly reduced in a concentration-dependent manner (Figure 8.1). At the highest concentration (100 ppm), the mean survival was 10 days (range 5 to 22 days) compared with 29 days (range 25 to 34 days) for individuals in the control group. Therefore, unexposed aphids live approximately 19 days longer than aphids exposed to 100 ppm Multineem®. At 10% SE, maximum longevity was 6 days, compared to 34 days for control. Maximum survival at 100 ppm and 10% SE were, respectively, 24 and 22 days versus 28 days for control.

When newly born nymphs were exposed to leaves treated with SE, no survival was observed. Five days after exposure, nymphs began to die in increasing numbers and by the day 7 100% mortality was observed in the SE treatment. The mortality in 10 ppm treatment did not change significantly between day 10 and day 20, but in 20 ppm the mortality increased to 50% by the day 15 (Figure 8.1). The difference became noticeable 4-6 days after the beginning of the treatment. The regression of mean percent mortality with treatment dosage suggests that, in general, the survival decreased as dosage increased in a linear fashion ($r = 0.940$, $P < 0.001$).

Fecundity was also greatly reduced after exposure to pea plants treated with the neem formulations. Significant differences ($P<0.001$) between the number of nymphs produced by treated and control adults were apparent, with the former nymphipositing more than twice as many nymphs as aphids treated with as low as 10 ppm Multineem®. When newborn nymphs were allowed to feed on pea plants treated with the aqueous neem seed extract, no survival to reproduction was observed. The highest rates of Multineem® also killed most of the nymphs before they reached adult stage. Minor effects were recorded on survival of adults and the number of offspring produced. However, when young nymphs were exposed to treated leaves, survival to adult stage and production of nymphs by the surviving adults was reduced in a dose-dependent manner. The average number of offspring that produced a female over a lifetime was 69.8 in the control group and only 3.4 in the group exposed to 100 ppm Multineem® from birth. Lethal concentrations of Multineem®, which resulted in a 50% reduction in survival of young nymphs (LC_{50}) after 12 d was 49.3 ppm, ranging from 31.6 to 81.6 ppm (Table 8.4).

Experiment 3. Pea aphids exposed to treated plants as adults. Both neem formulations also significantly ($P<0.05$) reduced longevity of adult aphids exposed to treated pea plants. When adult aphids were exposed to plants treated with different concentrations of the neem formulations, the survival and fecundity declined with increasing dosage (Table 8.3). The longevity was influenced by treatment rates and differences between the high rates (60, 80, 100 ppm, 5% and 10% SE) and low rates (10, 20, 40 ppm) were significant. Twenty-one and 29% reduction in longevity occurred in individuals exposed to the highest concentrations of Multineem® and SE. The longevity of treated adults ranged between 16.8 and 24.0 days in treatments, while it was 24 days in controls. In the 20 ppm or less treatments, minor effects were found on survival of adults. Although survival of *A. pisum* exposed as adults was reduced, reduction was much less than in individuals exposed as nymphs (Figure 8.2). Adults that were exposed to treated plants appeared normal, with no apparent color change as was noted in nymphs but they exhibited daily mortality than controls at the higher concentration (Figure 8.2). Adult mortality caused by exposure to neem appeared to stabilize approximately 20 days after exposure.

When adult aphids were exposed to treated plants, no acute toxicity was observed (Table 8.3). Their number of offspring also declined in response to pesticide exposure in the population exposed as adults. However, the reduction in progeny number was much less dramatic than aphids exposed to treated plants from birth (Figure 8.3). Significantly ($P<0.05$) fewer offspring were produced at the highest concentrations of Multineem® (80 and 100 ppm) and SE than other treatments. Rates up to 60 ppm had very little effect on the fecundity. Approximately 0.5 times more nymphs were produced by *A. pisum* control adults than by adults with 100 ppm. However, control adults were more than twice of that of 10%

SE. A mean of 101.7 nymphs were produced on control plants, compared with 73.1 for 100 ppm treated plants and 40.7 for plants treated with 10 % SE. Multineem® was even slower acting in adult *A. pisum* than in immatures. The LC_{50} 20 days after treatment for adults was 55.4 mg azadirachtin $liter^{-1}$, ranging from 28.4 to 125.6 mg $liter^{-1}$ (Table 8.5).

The fecundity of *A. pisum* exposed from birth and as adults is depicted in Figure 8.3. When the newborn nymphs were exposed to Multineem® treated plants, fecundity was reduced in a concentration-dependent manner and become close to zero at 80 and 100 ppm azadirachtin. This response indicates that if a population of *A. pisum* was exposed to Multineem® applied at 20 ppm azadirachtin from birth, the population would become extremely low. The fecundity for *A. pisum* exposed as adults were not affected by Multineem®.

Experiment 4. Contact toxicity. For comparison with the test mentioned in this section, topical toxicity data shown in Table 8.4 are presented as the concentrations applied to aphids, not the amount per aphid body weight or the amount per aphid. When applied topically, the neem formulations significantly ($P<0.05$) reduced longevity and fecundity of adult aphids (Table 8.4). Life spans of individuals treated with 100 ppm and 10% SE were respectively 29 and 40% shorter than those in control. The difference, however, was not significant among the lower concentrations and the control.

The number of offspring followed a similar trend to that of the adults exposed to treated plants. For *A. pisum* adults sprayed directly with neem solutions of various concentrations, differences in the number of offspring per female (Table 8.4) were significant ($P<0.05$). The reproduction rate of aphids exposed to all treatments (except 10 ppm) was significantly lower than the reproduction rate of the control. The mean number of progeny

produced per aphid exposed to 10% SE was significantly ($P<0.05$) lower than the mean progeny number of all the treatments. In this test the mean fecundity over the lifespan of an adult was 81.1 nymphs for control aphids, compared with 44.8 nymphs for 100 ppm and 25.1 for aphids sprayed with 10% SE (Table 8.4).

A similar analysis was conducted on adult aphids exposed to treated plants ($r = -0.871$, $P< 0.001$) and adults treated topically ($r = -0.952$, $P<0.001$). These significant relationships indicate that the high rates of neem may be an indicator of the extent of reduction in longevity that would be sustained.

Discussion

In a laboratory study neem seed extracts applied to broadbean (*Vicia faba* F.) plants killed the first-instars of pea aphid and black bean aphid (*Aphis fabae*) (Schauer 1984). Stark & Rangus (1994) and Stark & Wennergren (1995) also reported that a pea aphid population exposed to neem from birth was affected more than was the case when the population was exposed as adults, with the neem extracts causing molting abnormalities and reduced longevity and fecundity. Results of this study show that Multineem® influences the development of immature *A. pisum* by also disrupting molting, and reducing longevity and fecundity, thereby largely agreeing with the findings of these authors. However, the degree of efficacy of Multineem® and SE varied greatly, depending upon the life stage of *A. pisum* that was exposed, as well as the type of exposure. Although the neem preparations were very effective against *A. pisum* exposed from birth, this effects were much less pronounced against individuals exposed as adults. This is to be expected since azadirachtin, the major active ingredient in neem, is primarily a growth regulator (Schmutterer 1990) and therefore

affects insects during molting. Adult longevity and fecundity were both affected by neem preparations, but a reduction in fecundity seemed to be the major effect in adult *A. pisum.*

Rather than direct toxic effects, aphid mortality due to neem application resulted from the disruption of nymphal molts, a phenomenon that has already been demonstrated for several other insects (Schmutterer 1990; Isman, Koul, Arnason, Stewart & Salloum 1991; Lowery & Isman, 1994; Celal Tuncer & Almazee 1998). Mortality primarily results from insect growth regulating (IGR) activities of neem (Saxena 1989; Wood 1990) and aphids treated with neem in the adult stage would therefore be less susceptible to the extract. Schaur (1984) demonstrated that during molts of *A. pisum* nymphs feeding on broadbean plants treated with neem seed extract, the old nymphal cuticle began to detach from the body, but could not be ruptured, or that the cuticle ruptured, but could not be cast off completely.

Price & Schaster (1990) showed that earlier instars of sweetpotato whitefly, *Bemesia tabaci* (Genadius) (Aleyrodidae) were more susceptible to neem formulation Morgsan-O® compared with later instars, and that there was little mortality to eggs or adults. The present study has demonstrated that *A. pisum* is susceptible to the insect growth regulator activity of azadirachtin, with mortality occurring mainly during and as a result of failed attempts to molt.

Although reaction to neem by insects would result primarily from ingestion of the product, properly formulated materials possess some contact toxicity. Compared with synthetic neurotoxins, neem does not provide rapid control and at 17°C at least 9 days may be required for the complete expression of the IGR effects (Lowery & Isman 1994). Some of these results are similar to those of Stark & Rangus (1994), which state that reductions in longevity, number of molts and fecundity were concentration-dependent. Differences that do

exist between the two studies could account for the observed response differences of *A. pisum* to neem.

The results of the present study indicate that low concentrations of Multineem® are effective inhibitors of adult pea aphid emergence from treated field pea plants. Furthermore, individuals that survive at the first molt, still die at higher rates than the controls. *A. pisum* nymphs that survive treatments and emerge as adults also exhibit a reduced ability to nymphiposit. The reduced fecundity and survival of *A. pisum* when exposed to treated plants continuously is comparable with the results of Stark & Rangus (1994), who observed increase of mortality and reduced fecundity of aphids when exposed to the neem formulation Morgosan-O.

Other species of aphids, including the green peach aphid, *Myzus persicae* (Sulzer), currant-lettuce aphid, *Nasonovia ribisnigri* (Mosley), and strawberry aphid, *Chaetosiphon frageafolii* (Cockerell) are also susceptible to neem under both laboratory and field conditions (Lowrey, Isman, & Brard 1993). According to these authors the contact toxicity of neem did not contribute significantly to the reduction in the numbers of these three aphid species and the respective host plants each had a major contributing effect on mortality. Foliar applications of neem seed oil or neem seed extract have also been shown to effectively control *Aphis gossypii* on cotton (Siddig 1987), demonstrating that neem-based insecticides are potentially very effective as aphicides. The deleterious effects of neem on offspring production have been recorded in insects other than aphids, including stored product pests. Xie, Fields & Isman (1995) showed that the offspring production in *Cryptolestes ferrugineus* (Stephens) (Laemophloeidae) and *Tribolium castaneum* (Herbst) (Tenebrionidae) was negatively affected by neem treatments and that the response was proportional to the

concentration of azadirachtin in the solution. Similarly, Musabiyamana, Saxon, Kairu, Ogoi & Khan (2001) reported that significantly fewer eggs were laid and more larvae died when the banana corm borer, *Cosmopolites sordidus* (Germar) (Curculionidae), was confined to neem treated pseudostems for 14 days.

Stark & Rangus (1994) reported that in a residue bioassay of first instar nymphs of *A. pisum*, the seven day LC_{50} was 27.5 mg azadirachtin liter^{-1} (95% FL; 22.8 – 32.5), which was less by 21.8 mg (95% FL; 31.6 – 81.6) than was found in the present study 12 days after treatment. LC_{50} values for adult aphids sprayed and reared on plants treated with azadirachtin also varied greatly to that of Stark & Rangus (1994). The variability may have resulted from differences between aphid clones or from differences in the penetration and translaminar movement of azadirachtin into the various host plants. Lowery & Isman (1994) demonstrated that the LC_{50} value for *Chaetosiphon fragaefolii* on strawberry (635 ppm azadirachtin) is more than nine times that of *Fimbriaphis fimbriata* Richards (69.1 ppm azadirachtin) on the same host. Previous studies also indicated that the efficacy of neem is influenced by the host plant. For example, the LC_{50} value for the second nymphal instar of *M. persicae* on corn was more than 20 times higher than that on mustard cabbage (Lowery & Isman 1994). Foliar applications of neem seed oil were also more efficacious for *M. persicae* on pepper than on rutabaga (Lowery *et al.* 1993), and soil drenches with Morgsan-O®, a neem-based insecticide, reduced the number of leafhoppers on marigold and chrysanthemum, but not on zinnia (Jacobson 1990).

In conclusion, the data of this study shows that neem preparations were very effective against immatures of *A. pisum*. However, the seed extract at 5% and 10% aqueous solution was very effective for adult aphids sprayed directly and reared on plants treated with the

solution. The long-term deleterious effects of Multineem® on longevity and fecundity of adult aphids and the reduced nymphal survival at concentrations as low as 20 ppm were impressive. It is clear from the results of this study, and other reports, that neem insecticide deserves serious consideration for inclusion in a field pea IPM program.

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Table 8.1 Number of *Acyrthosiphon pisum* on plants exposed to neem at different concentrations of 'Multineem®' and seed extract, Holetta, Ethiopia 2000.

Treatment	Final number of aphids (± SD)*
0 mg azadirachtin/l	561.0 ±52.5a
10 mg azadirachtin/l	532.3±40.2a
20 mg azadirachtin/l	488.2±51.3b
40 mg azadirachtin/l	449.8±44.3bc
60 mg azadirachtin/l	443.1±63.2cd
80 mg azadirachtin/l	410.5±58.0d
100 mg azadirachtin/l	352.6±28.8e
5 % SE	263.4±30.2f
10 % SE	212.1±30.9g
CV (%)	11.5

* Means within a column followed by the same letter are not significantly different (P= 0.05); DMRT option of MSTAT-C ANOVA procedure (1990).

Table 8.2 Effects of neem insecticide formulations on *Acyrthosiphon pisum* exposed as newborn nymphs to treated field pea plants, Holetta, 2000.

Treatment	No. of molts (± SEM) *	Longevity (± SEM) (days)	Number of offspring (± SEM)
0 mg azadirachtin/l	4.0 ±0.0a	29.4±2.9a	69.8± 9.3a
10 mg azadirachtin/l	3.9 ±0.3ab	24.8±6.4ab	29.0± 4.3b
20 mg azadirachtin/l	3.6 ±0.4abc	19.9±9.2bc	15.8± 3.1c
40 mg azadirachtin/l	3.1 ±0.8cd	14.4±8.5cd	9.9± 2.3cd
60 mg azadirachtin/l	3.2 ±1.1bcd	14.1±7.9cd	9.0± 2.6cd
80 mg azadirachtin/l	2.7 ±1.3d	11.9±7.1d	4.7± 1.4d
100 mg azadirachtin/l	2.6 ±0.6d	10.2±5.1d	3.4± 1.2d
5 % SE	0.5 ±0.7e	4.2±1.3e	0.0± 0.0d
10 % SE	0.7 ±0.8e	4.1±1.7e	0.0± 0.0d
CV (%)	15.2	22.1	52.5

* Means within a column followed by the same letter are not significantly different (P= 0.05); DMRT option of MSTAT-C ANOVA procedure (1990).

Table 8.3 Effect of neem insecticide formulations on adult *Acyrthosiphon pisum* exposed to treated field pea plants, Holetta, 2000.

Treatment	Longevity (± SEM)[a] (days)*	Number of offspring (± SEM)
0 mg azadirachtin/l	23.7±3.1**ab**	101.7±10.9**a**
10 mg azadirachtin/l	24.2±3.2**a**	97.8±8.8**ab**
20 mg azadirachtin/l	21.5±3.0**abc**	93.2±12.0**ab**
40 mg azadirachtin/l	21.7±1.9**abc**	91.3±8.2**ab**
60 mg azadirachtin/l	21.0±2.1**bcd**	89.6±17.4**ab**
80 mg azadirachtin/l	21.0±3.1**bcd**	84.4±9.8**bc**
100 mg azadirachtin/l	19.0±3.8**cd**	73.1±13.7**cd**
5 % SE	18.5±2.3**de**	67.9±14.6**d**
10 % SE	16.8±2.6**e**	40.7±15.0**e**
CV (%)	13.6	17.3

* means with out letters in common differ significantly, (P= 0.05); DMRT option of MSTAT-C ANOVA procedure (1990).

Table 8.4 Effects of topical application of neem insecticide formulations on adult *Acyrthosiphon pisum.* (Holetta, 2000).

Treatment	Longevity (± SEM) (days)*	Number of offspring (± SEM) *
0 mg azadirachtin/l	23.7 ± 1.9ab	81.8 ± 9.2a
10 mg azadirachtin/l	25.1 ± 3.1a	79.9 ± 11.9ab
20 mg azadirachtin/l	24.1 ± 3.6ab	69.6 ± 5.7bc
40 mg azadirachtin/l	22.6 ± 2.7ab	70.6 ± 10.6bc
60 mg azadirachtin/l	21.0 ± 4.2bc	68.8 ± 13.5c
80 mg azadirachtin/l	18.6 ± 3.6cd	58.0 ± 11.3d
100 mg azadirachtin/l	17.0 ± 4.9de	44.8 ± 15.1e
5 % SE	17.4 ± 4.5de	41.5 ± 9.3e
10 % SE	13.7 ± 3.2e	25.1 ± 7.3f
CV (%)	18.14	18.66

* Aphids were treated with 0.59 µ l of solution
* means with out letters in common differ significantly, (P= 0.05; DMRT option of MSTAT-C ANOVA procedure (1990).

Table 8.5 Toxicity of Multineem® to *Acyrthosiphon pisum* reared on field pea as newborn nymphs or adults or applied topically, Holetta, 2000.

Stage initially exposed	Day	Slope (SE)	LC50 (95% FL) mg azadirachtin liter^{-1}
Nymphs	12	1.85 (0.21)	49.29 (31.58 – 81.60)
Adults	20	1.33 (0.31)	55.54 (28.39 – 125.60)
Adults (topically applied)	20	2.14 (0.31)	60.2 (39.8 – 102.5)

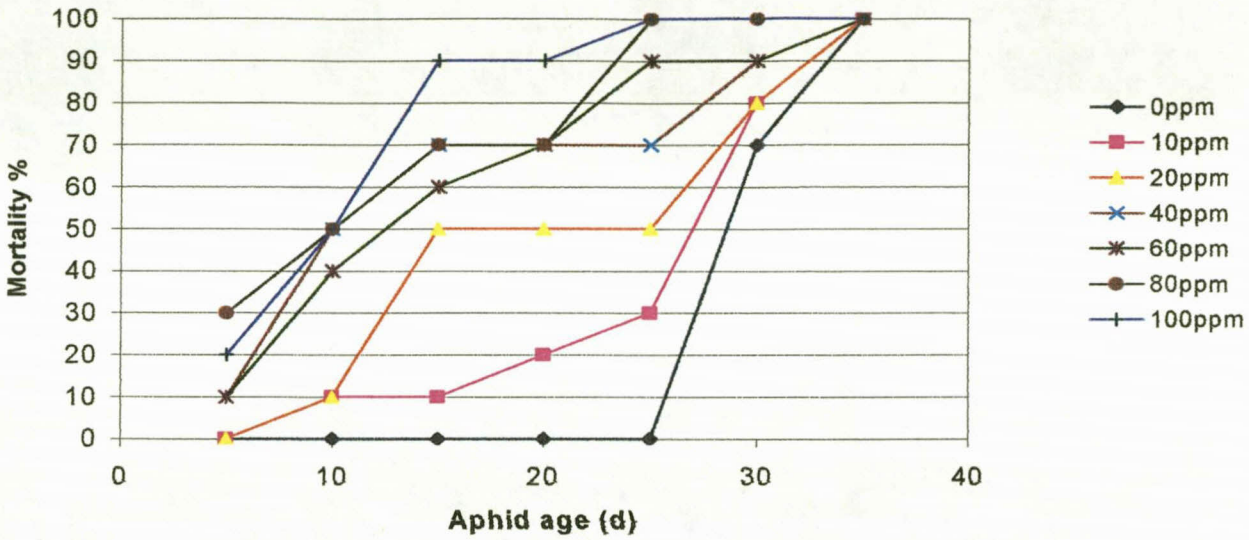

Figure 8.1 Mortality of *Acyrthosiphon pisum* nymphs exposed to the Multineem® treated plants from birth, Holetta, 2000.

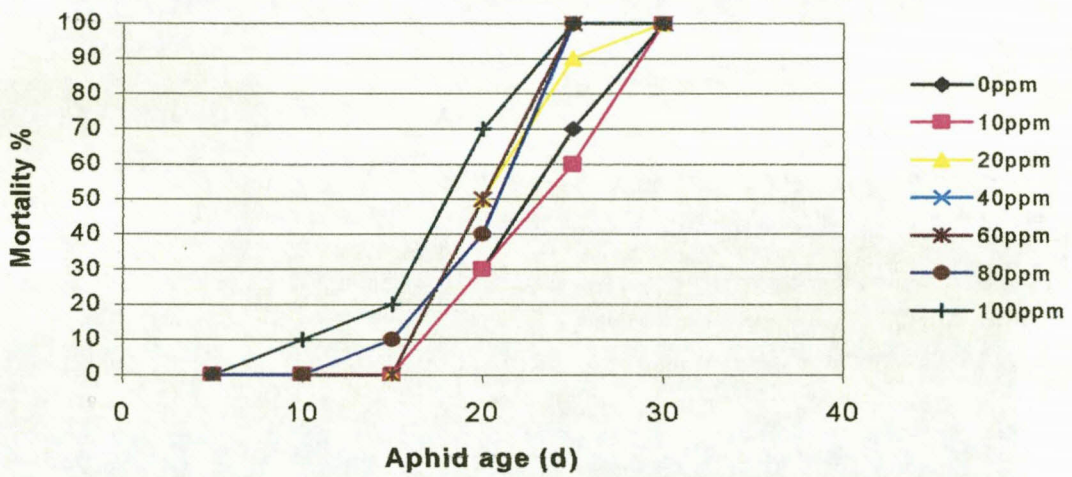

Figure 8.2 Mortality of *Acyrthosiphon pisum* exposed to treated plants of Multineem® as adults, Holetta, 2000.

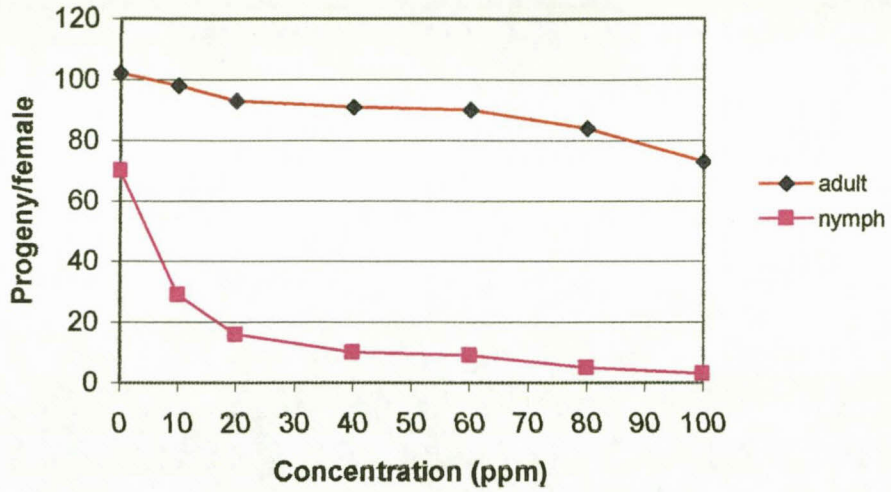

Figure 8.3 Fecundity of *Acyrthosiphon pisum* exposed to different concentrations of Multineem® from birth and as adults, Holetta, 2000.

CHAPTER 9

Recommendations for Integrated Pest Management in Field pea (*Pisum sativum* L.)

Growing concern over the impact of agrochemicals on food safety and the environment necessitate that integrated pest management (IPM) be practiced with minimum reliance on pesticides. This approach also takes into consideration the pest control needs of the resource-limited farmers in most developing countries, particularly in Africa, by emphasizing biointensive pest management (BPM). Within this framework, host plant resistance, compromising the use of pest tolerant cultivars forms the focus and is supported by other pest management components. These are, amongst others, biological control (deploying predators, parasitoids and microbes as control agents), botanical (natural) pest control (the use of derivatives of locally available plants with pest control properties) and cultural control and supportive tactics (such as pest monitoring, loss assessment and establishment of economic threshold levels). Control by means of synthetic pesticides is only used as a last resort when other strategies fail to curb pest eruptions.

IPM must reflect and reinforce the goals of a more sustainable agriculture. Over the long term sustainable agriculture enhances environmental quality and the natural base on which agriculture in general depends. From this, basic human nutrition and fiber needs are met, economic viability is possible and the health and quality of life for farmers and society as a whole is improved.

A long-term economic solution to the pea aphid (*Acyrthosiphon pisum*) pest problem on field pea (*Pisum sativum*) is only possible through the integration of the control strategies as mentioned above. Based on information attained from the preceding chapters, this final

chapter therefore deals with the composition of an integrated pest management strategy that takes into account the needs and circumstances of individual farmers, be it for commercial farming or low-input small-scale farming. Farming systems differ widely in the area and type of land farmed, cropping systems, wealth and farming objectives. The objectives of subsistence farmers, for instance, are generally held to be vastly different from those of commercial farmers. Overall however, farmers are concerned with generating a food supply on a profitable basis.

Since synthetic, and to a certain extent natural insecticides, will probably remain the key factor in management for commercial farmers in the foreseeable future, a chemically intensive IPM program is proposed. This program is based on the judicious use of environment-friendly insecticides and cognisance of economic threshold levels (ETL), supplemented by host plant resistance and cultural control methods whenever possible. Previous research in this regard has indicated that timely and correct application of insecticides could result in substantial yield increases of the crop concerned.

Information for pesticide use should be based on sampling during critical periods of crop development, and treatment decisions should be based on pest densities or severity, so that unnecessary treatments are avoided and maximum ecological selectivity is encouraged. Timing of insecticide application in the commercial farming environment should be determined by ETLs. In field pea ETL of 35% - 40% infested plants has already been established for susceptible plants and 40% - 45% infested plants for tolerant plants. Depending on whether yield potential justifies one or two insecticide applications, different chemical strategies may be considered. In the case of low to moderate pea aphid infestations, a single insecticide spray should be applied during the flowering and early pod stage of plant

development. Since *Acyrthosiphon pisum* infestations proved to be greater in later plantings, plantings done late will also need to be chemically protected during the vegetative stage of plant development. In cases where yield potential warrants a follow-up application, this application must be done during the pod stage. More than two sprays are not justified for control of pea aphid on field pea. It is important to note that insecticide application at early plant growth stages will not necessarily protect plants against reinfestation and the possibility for a follow-up application always exists.

The use of resistant host plants has several advantages in commercial farming systems, in that it leads to a direct reduction in yield loss and may lead to increased pesticide efficacy (Chapters 2, 3 and 4). Nevertheless, several factors should be taken into account before deciding on the use of resistant cultivars. These include the yield loss of existing cultivars due to pea aphid damage, the frequency and severity of losses and what control measures other than plant resistance are available as cheap and effective short-term solutions. In order for a pea aphid and ascochyta blight resistant cultivar to be accepted by farmers, it should yield more than the susceptible cultivar under attack of the pest, and at least as much as the susceptible cultivar in the absence of the pest. Host plant resistance would be of significant value in areas where the pest populations frequently occur at high levels, or during epidemic years. Under such circumstances even lower yielding pest resistant cultivars may be accepted.

Varieties with low to moderate levels of resistance against pea aphid and ascochyta blight (Chapters 3 and 4) can be very useful for pest suppression over a period of time. The adverse effect of resistant genotypes on pest populations are continuous, commutative and of no cost to the farmers. It should also be borne in mind that low pest population density

resulting from plant resistance can assist in pest management by means of natural enemies and reduce the number of pesticide treatments that may be needed. A reduction of the rate at which the pest populations increase will delay the pest from attaining an economic threshold level. This is especially true if the resistance mechanisms increase the mortality of the immature stages and disrupt the developmental period of the survivors. For example, lines selected in preliminary screening trials (Chapter 2) demonstrated the value of these phenomena when compared to susceptible lines (Chapter 3).

Unfortunately, the current levels of resistance in commercial field pea cultivars are low and tolerance in some cultivars seems to be the only resistant component for current use in pest management (Chapters 3 and 4). Plant resistance that only depends on non-preference may not operate under no-choice conditions or under situations of high pest density. The planting of pest tolerant cultivars, followed by timely and effective pesticide applications, should result in improved yields and cost-benefit ratios of control. However, while tolerance to pest damage may result in reduced yield losses and higher economic returns, it may, in the long run, underscore the pest problem, since tolerant cultivars do not adversely affect the biology of the pest. Future research should therefore focus primarily on the development of field pea cultivars with acceptable levels of antibiosis resistance.

Cultural control practices can have a profound influence on insect and disease survival, their persistence in a particular environment and damage levels on the crop, and each practice needs to be seen as part of the total crop production system. The development of a sound pest control strategy requires an understanding of the principles underlying the fluctuation of populations that make up the ecosystem. Population densities of pests generally fluctuate significantly under monoculture conditions and are more stable under

polyculture conditions. From the viewpoint of pea aphid and ascochyta blight management, it would be desirable to rather practice intercropping than monoculture. Intercropping is a common practice in many parts of the world, particularly in small-scale farming in the developing world. Overall, the development of an IPM model for resource-poor farmers in developing countries should be geared towards maximizing the use of safe, cheap and simple pest management methods, incorporating those traditionally used by third world farmers and integrating them with the use of the safest possible chemical pesticides when necessary. In this context, the intercropping strategy employed by most resource-poor farmers in third world countries has several advantages. These include (1) a reasonable level of insurance against crop failure, (2) provision of a steady food supply which provides an assortment of food types, (3) provision of continuous soil cover which serves as an important soil conservation element, and (4) provision of certain meaningful crop combinations, e.g. field pea and mustard which gives a total yield per unit area which is greater than when field peas are grown separately as a single crop (Chapter 6). Intercropping, moreover, helps in the reduction of insect population numbers and disease severity. Field pea mixed with, for instance, mustard proved to reduce pea aphid population numbers and the severity of ascochyta blight which resulted in a land equivalent ratio exceeding 1.0, indicating that the mixed system combination is efficient in terms of yield (Chapter 6). However, the factors responsible for this increase in crop yields within intercropping systems is still an issue in need of detailed research both in Africa and elsewhere.

Small-scale farmers in developing countries are already practitioners of IPM and the strategy should, therefore, not necessarily be regarded as a new technology. By and large, farmers in Africa grow small patches of several crop mixtures in one field, and monoculture

cultivation is relatively rare. This approach is believed to create heterogeneity and diversity, which may impose a physical or chemical restraint on a potential pest (disease and/or insect). Perhaps this may be an explanation for the rare occurrence of pest outbreaks (except for migratory pests such as the desert locust and the African armyworm) in subsistence agriculture, when compared to the regularity of pest outbreaks in commercial agriculture. What is therefore required is to provide a scientific basis to what the farmers already know by devising technologies that can supplement existing knowledge. The key issue underlying all this is whether a rapidly increasing human population and acute food shortage in Africa is adequately provided for by a subsistence agricultural system? The answer is that there is not adequate provision, largely due to increased population pressure. This has subsequently prompted many African governments to "modernize" agriculture, based on the "green revolution" model. Technologies such as irrigation, fertilizer, and pesticides are used as a package to control the environment and grow high yielding varieties. This is a striking contrast to developed countries where human population numbers have stabilized and where there are larger yields from smaller areas of cropped land. Arable agriculture in developed countries, however, still remains dependent on pesticides and IPM is mostly implemented to reduce and refine pesticide use so as to minimize harm to natural enemies and the environment.

Time of planting and fertilizer application is also reported to have a pronounced influence on pea aphid density and ascochyta blight infection (Chapter 7). The planting of moderately resistant genotypes at adjusted planting dates in order to escape major pest and disease infestation can be valuable in limiting damage to the crop. Resistant genotypes should in the future be made available to small farmers in order to increase the number of

mechanisms at their disposal for managing pea aphid and ascochyta blight infection. The effect of the current levels of resistance should therefore be supplemented by the use of cultural control methods.

The numerous cultural control methods that exist for field pea protection are simple and easy to follow, but they are subject to the variability of geographical zones and weather patterns. Cultural control practices are intended to disrupt or slow down the population build-up of pea aphid and ascochyta blight in field pea. Exploitation of behavioral and epidemiological characteristics of pea aphid and ascochyta blight through practices such as intercropping of field pea and mustard plants (Chapter 6) and adjustment of planting dates and fertilizer application (Chapter 7) can also result in reduced infection levels.

Although biological control agents are effective in suppressing pea aphid numbers, their numbers are often not reduced below economic injury levels (EILs). Farmers can therefore not confidently rely on biological control to limit pest damage. However, since the reduced effect of biological control agents may partly be ascribed to the indiscriminate use of insecticides, the implementation of softer botanical insecticides (*e.g.* neem) (Chapter 8), may alleviate the constraints inflicted on these agents and lead to an increase in their abundance and efficacy. Selective chemical control of pea aphids can be achieved with some botanicals, of which neem has proven most promising. In Ethiopia, neem has to be applied before flowering to act against early migrating aphids, while one treatment shortly after bloom is usually sufficient to control the remaining populations. High aphid densities around flowering time causes serious damage. Pirimor and neem extracts are both regarded as selective chemical management agents, causing minimal side effects to natural enemies and can therefore be considered compatible to biological control agents in integrated control

systems. In addition, the selectivity and the novel chemistry of neem-based products make them important candidate insecticides in an IPM program for pea aphid where resistance to conventional insecticides has constantly been a widespread problem. Based on its relatively minimal effect on natural enemies, its high efficacy in pea aphid control and its favorable cost-benefit ratio relative to other synthetic insecticides, the use of neem insecticides should therefore be promoted.

It should also be taken into account that the limited crop value in traditional farming systems rarely justifies the use of pesticides. Therefore, the main focus of developing and implementing IPM in Africa will not be reducing pesticide usage, but rather to build IPM programs around the traditional pest management approaches that abound in small-scale agriculture on the continent.

Owing to the high EILs that apply to low-input farming conditions, biological control is especially important in small farming systems. The success rate of parasitoids and predators can even be increased in cases where resistant host plants and cultural control practices are employed as primary pest management tools, since these IPM components are favorable to parasitoid and predator establishment. Investigations into the potential of implementing biological control on pea aphid by using *Hippodamia variegata* (Coleoptera: Coccinellidae) and *Beauveria bassiana* (Hyphomycetes) under field conditions indicated that the predator reduced aphid population growth and increased yield (Chapter 5). The use of indigenous predators as biological control agents should therefore be promoted, the reason being their environmental friendliness and simple mass-rearing capabilities. The implementation of the biological control strategy developed in this study (Chapter 5), in

conjunction with resistant cultivars, may contribute towards the alleviation of the pea aphid pest problem and increase field pea production.

Few pathogens are being exploited in tropical countries, probably because very little research in this area has been undertaken. Some argue that the germicidal effect of sunlight in the tropics may reduce chances of success, compared to that in temperate zones, in the case of some entomopathogens, e.g. *B. bassiana*. In this study, for example, the field experiment showed that *B. bassiana* had very little effect on pea aphid populations probably because of the prevailing prolonged dry and sunny weather (Chapter 5).

The success of an IPM program requires a certain degree of knowledge, expertise, infrastructure, research and community organization and these criteria are often lacking or inadequate in many developing countries. Both basic and applied research on the interaction between pests, their natural enemies and their host crops are essential. The knowledge of farmers regarding their land and the agriculture practices and pest control methods should form the basis on which the IPM program is devised. Indeed, a method that incorporates the traditional practices of the farmer is far more likely to be accepted by farmers, than would a switch to a completely new system of pest control. Past experience seems to indicate that in many instances the development of technological packages has failed to take into account realities in the field and the true needs of the farmers.

Experience in Africa has also shown that it is difficult to persuade subsistence farmers to accept a costly technological package, even if it will generate them maximum profits. Rather than adopt new management strategies that are bound to maximize profits in the long term, farmers are more inclined to minimize profits in the process of providing for survival of their families in the short term.

Systems of IPM are now widely accepted by being desirable strategies for sustainable crop protection. Properly implemented, they should provide sustainable crop protection systems which centers on environmental concerns and the need for conservation of biological diversity. Field pea, together with the pests which attack it, requires detailed research in different regions in order to formulate a workable, practical and realistic IPM program. Coordinated research efforts to date into IPM in field pea is lacking.

The different field pea production systems in Ethiopia specifically are each characterized by its infrastructure and farming practices. These differences necessitate a pesticide based IPM approach to commercial farming and a host plant resistance / cultural control based approach in low-input, small farming systems. Moreover, the implementation of these research findings from this study, especially those regarding biological control and the exploitation of host plant resistance, hold many advantages, both in commercial and small scale farming systems. Future research should give high priority to breed for field pea lines that have a combined resistance to both pea aphid and ascochyta blight. This would provide a useful cultivar for subsistence farmers in Ethiopia, who are unable to afford chemical control measures.