MANAGEMENT OF RUST OF FRENCH BEANS BY SEED TREATMENT AND DETERMINATION OF YIELD LOSS IN RELATION TO THE STAGE OF INFECTION

BY

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A thesis submitted in partial fulfilment of the requirements

for the degree of

MASTER OF SCIENCE

IN

PLANT PATHOLOGY

DEPARTMENT OF CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI, KENYA

1998

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This thesis is dedicated to my grandparents Muhoi wa Wairagu and Mwihaki wa Muhoi and to my dear mother Wanjiku wa Mwangi.

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ACKNOWLEDGEMENTS

I would like to thank the University of Nairobi through The Dean Faculty of Agriculture who enabled me to study through a scholarship. I thank GTZ ipm Horticulture who provided the funds for the research work and also liaised with other organisations who had an interest in the work. I thank the management of Homegrown (K) Ltd especially the manager at Marula farm Mr. Aimie Gakirage who provided their farm, machinery and also labour for the part of research carried out at Naivasha. I thank the staff at Rhone Poulenc (K) Ltd and Bayer E.A Ltd who responded and availed free chemical samples for evaluation in the trials.

I thank my supervisors Dr.E.W.Mutitu who supervised me at each step of the work, Prof.D.M.Mukunya who found time from his busy schedule to look at my work and Dr.A.A. Seif who especially was important in my link with GTZ. Their comments, criticisms and encouragements have made this work what it is today.

I thank Mr. Musyimi. A. B and all the staff at the Department of Crop Science who helped me all along during my study. Many thanks also go to my colleagues Wakahiu, Wahome, Kigamwa and Sande who were a challenge and an inspiration to me by the commitment they had to their work. I thank my friends Muthoni Mwaura, Watiri Haru, Wangui Murage and all others who stood by me either in prayer or other means all through this work.

Thank you all.

ABSTRACT

Uromyces appendiculatus var *appendiculatus*, the causal agent of rust, is a serious pathogen of beans wherever they are grown in the world. Studies on various aspects of the disease were carried out at the Kabete University farm and at Homegrown farm, Naivasha between January and September 1996.

Results obtained showed that the bean rust fungus is capable of reducing yield by up to 35 %, depending on the stage at which infection occurs and the prevailing weather conditions. The maximum effect on yield was realised if the beans were infected between the third trifoliate leaf and the pre-flowering stage of growth.

Efficacy of six fungicides as seed dressings against the bean rust fungus was evaluated under both field and greenhouse conditions. Three of the fungicides namely Anvil, Baycor and Saprol are regularly used as foliar applications with known systemic action on bean rust. The others were Rakil 025, Rakil 040 and Real of which none had been used against bean rust before. No compound was found to be effective when germination occurred after germination.

In another fungicide screening trial, Anvil sprays significantly reduced disease levels when applied early at the third trifoliate leaf stage. Cell suspensions of two isolates of *Bacillus* sp were also evaluated for biological control of the rust pathogen. The suspensions were effective in reducing spore germination under laboratory conditions and also suppressed the development of rust on beans under greenhouse conditions. However under field conditions where disease pressure was much higher the

suspensions performed poorly. Suspensions of one isolate had a significant effect and reduced rust infection during the first one week after application. However the effect was offset after some pustules established on the leaves and infection increased.

CHAPTER ONE: INTRODUCTION

Bean (*Phaseolus vulgaris* L) ranks second to maize as a food crop in Kenya.The terms French bean, snap bean, kidney bean and pole bean are used synonymously for beans that produce seeds for vegetables (Njeru, 1989).In the export market such terms as Haricot verts, Fillet beans or string beans refer to French beans (HCDA,1996).

In Kenya the crop is grown mainly for the fresh export markets especially to France and the United Kingdom. Local consumption of French beans has also increased over the last few years (HCDA,1996). French beans in Kenya are produced by both large scale and small scale producers. Cultivation is mainly in Central province, Eastern, Western and to a lesser extent in Coast province especially Taita Taveta (Njeru,1989). The most widely grown variety is Monel. It is grown mainly for the fresh export market and to a lesser extent for canning. Newly introduced varieties include Claudia, Gloria, Maasai, Morgan and Espanda (HCDA,1996).

1.1 IMPORTANCE OF FRENCH BEANS IN KENYA

The benefits derived from French beans are both nutritional and economical.Data available shows that French bean exports have been catering for a large share of the fresh export market in recent years. Export share was 23.9, 20.9 and 17.2 % in 1993,1994 and 1995 respectively. As at May 1996 fine grade exports were 2.77 M tons. This represented a 50.2 % increase when compared to exports for the same period in 1995 (HCDA,1996). An increasing proportion of the urban population is consuming French bean pods

as part of their daily vegetable intake.

1.2 PROBLEMS IN PRODUCTION

The major problems are lack of Potassium fertilizers , proper storage facilities and also management of disease and pests (Njeru,1989; Jansen,1992). These problems are compounded by poor seed quality and distribution in addition to fluctuating producer prices due to undefined marketing structure.

1.2.1 INSECT PESTS

Major pests include American bollworm (*Heliothis armigera*), Red spider mite (*Tetranychus telarius*), Cutworms (*Agrotis* sp), beetles (*Astyllus* sp), caterpillars (*Rutellinae* sp) and bean fly (*Euphomyia* sp).

Nematodes especially *Meloidogyne* sp can be damaging to roots where they cause galls. Their injuries also predispose plants to secondary attack by fungal and bacterial pathogens.

1.2.2 DISEASES

Major foliar diseases include bean rust caused by *Uromyces appendiculatus* Pers, Arthracnose *Colletotrichum lindemuthianum* (Sacc. and Magn.) Angular leaf spot *Phaeoisariopsis griseola* (Sacc) Ferraris, Web blight *Thanatephorous cucumeris* (Frank) Donk and Aschochyta blight *Aschochyta* sp. Major root rots experienced are Fusarium root rot *F. solani* f.sp. *phaseoli* (Burk.) Snyder and Hansen, Fusarium wilt (yellows) *F.oxysporum* Schelect f.sp. *phaseoli* Kend. and Snyder and Rhizoctonia root rot *Rhizoctonia solani* Kuhn. Halo blight *Pseudomonas syringae* pv *phaseolicola*

(Burk) Young *et al* and *Xanthomonas campestris* pv *phaseoli* (Smith) Dye are commonly found as seed contaminants (Aart and Martial,1987;Ryall and Lipton,1978)

1.3 BEAN RUST

Bean rust is caused by *Uromyces appendiculatus* Pers (Unger) G.Wint (Syn) *Uromyces phaseoli* (Pers) G.Wint. It occurs worldwide but it is most prevalent in humid tropical and subtropical areas (Baker *et al*,1985; Robert,1991). Heavy rust attacks can destroy a crop almost completely or at least severely degrade the seed that is produced (Howland and McCartney,1966). In 1981 losses incurred on both dry and snap beans in two states in the USA were 78 and 54 % respectively (Baker *et al*,1985). A rust severity rating of 0.7 - 31.6 pustules /cm^2 had a matching yield loss of 336 - 1233 kg/ha representing potential losses of 13-54% (Venette and Jones,1982). In the export industry an even higher loss is incurred or anticipated due to rejected produce bearing high chemical residue levels (Ken *et al*, 1987).

1.3.1 DISEASE CONTROL

In Kenya and elsewhere in the world the tendency is to rely heavily on chemicals for the control of pests and diseases. Various chemicals have been evaluated and reported to be effective against bean rust (Bazirake,1974; Opio,1979; Ken *et al*,1987; Singh and Musyimi,1980).

Biological control has been reported by Baker *et al* (1983,1984,1985) using strains of *Bacillus subtilis* (Ehrenberg) Cohn and by Spencer (1980) using *Verticillium lecanii*. Cultural

control involving crop rotation, field sanitation and adjustment of planting dates has been suggested as a supplementary method of control (Robert, 1991).

For any control measure to be effective, it should be applied at the most critical time of infection. Lack of information about the critical stages of intervention, amongst other reasons has led to the abuse of chemicals (Juan and Pedro, 1992).

From the foregoing it can be seen that an Integrated Pest Management (IPM) strategy which uses all available methods to reduce pest populations below economic thresholds is desirable. There is little information on the use of IPM on snap beans (Cesar and Corrales, 1992). Presently there are no reports on the relationship between yield loss of French beans and the stage of infection by rust. This study was therefore undertaken to address the issue and also to evaluate some systemic fungicides for effect against bean rust when used as seed dressings.

1.4 OBJECTIVES OF THE STUDY

The study had the following objectives:

1. To evaluate the effect of seed treatment with systemic fungicides on ;
   (a) delaying infection of the growing seedling by the rust fungus.
   (b) the rate of disease progress after infection has occured.
   (c) yields obtained under field conditions.
2. To evaluate the effect of some recommended systemic foliar

fungicides applied at different stages of growth on the development of rust and on yield obtained under field conditions.

3. To quantify the losses incurred in French bean yields in relation to the stage of infection.
4. To evaluate the efficacy of cell suspensions of two *Bacillus* sp isolates for possible antagonistic activity against bean rust in the greenhouse and in the field .

CHAPTER 2: LITERATURE REVIEW

Beans (*Phaseolus vulgaris* L) belongs to the leguminoseae family which has about 600 genera and a corresponding number of species. Only about 40 of those species are of economic importance for human consumption (Hawthorn, 1981). The bean is of new world origin, principally South America (Kaplan, 1981). *P.vulgaris* is the best known and most widely cultivated species of *Phaseolus* in the world (Tripath and Singh, 1968). In the wild state bean or its near relatives are found from the lowlands of warm humid tropics to the cold high altitude mountains and hot deserts.

However, most familiar varieties grown in the tropics perform well with a relatively narrow ecological zone ranging 0-1000 m above sea level (Tindall, 1983). Many hundreds of cultivars are cultivated for immature pods and green or dry seeds. There is, however, no clear distinction between cultivars for pods and those for seeds, and the same cultivar may be used for both pods and seeds (Grubben, 1977). French beans are principally grown for immature pods which are consumed fresh or processed and canned.

The two major types grown are:

(a) Dwarf or bush cultivars which are day length neutral, early maturing, 20-60 cm in height, with lateral terminal inflorescence and determinate growth. These do not require any support.

(b) Climbing or pole cultivars with indeterminate growth, grow up to 3 m in height and are normally supported. Both day length neutral and short day length types are available.

French bean pods are thin and narrow, mostly straight or

curved, with colours ranging from yellow to dark green. The seeds also vary in colour but from white to black (Tindall, 1983). French beans can grow in a wide range of soil types, from light sand to clay, but grow best on friable, well drained medium loam soils with high organic matter. In poorly drained soils, like the heavy clays, germination may be poor. French beans as any other bean cultivar are also very sensitive to waterlogged conditions. The optimum soil pH is 6.5 - 7.5. However French beans tolerate a low pH of about 4.5 - 5.5, below which plant growth is impaired (HCDA, 1996). Below pH 4.5 they suffer aluminium, manganese and phosphorus deficiency (Tindall, 1983). In Kenya they are grown in pH range below 6.5 (Anon, 1989). French beans do well in agro-ecological zones which fall within the lower midland to lower highland zones.

Moderate rainfall of 900 -1200 mm p.a. is adequate if well distributed within the growing season. Heavy rainfall adversely affects flower fertilisation. The optimum altitude for growth falls between 1500 - 2100 m above sea level (HCDA, 1996). At higher altitudes the growth period is slightly prolonged (Njeru, 1989). In a study in Phillipines to compare the differences between lowland and highland French bean cultivation, Hermiria (1992) reported that 92 % of the farmers in the highlands had to use fungicides to control stem rot and rust whose occurrence seemed to be favoured by the colder, rainier environment.

French beans are also susceptible to damage by frost or excessive heat (Njeru, 1989). The optimum temperature range is 14 - 22°C (HCDA, 1995). Below 10°C the plants are destroyed by frost while above 30°C blossom drop is very serious and seed set

may be hampered (Anon, 1989).

2.1 IMPORTANCE OF FRENCH BEANS PRODUCTION

Nutritional value

Other than the use of pods as vegetables mature seeds and young leaves are used as food (Tindall, 1983). The pods are rich in minerals like Calcium, Phosphorus and in vitamins especially ascorbic acid (Nengel, 1979). The average nutrient content of French beans (per 100g edible portion) as recorded by Tindall (1983) are 88g water, 36 kcal, 2.5g protein, 0.2g fat, 7g CHO, 1.8g fibre, 43mg Calcium, 48mg Phosphorous, 1.4mg Iron, 750 equivalents B-carotene, 0.8mg thiamine, 0.12mg Riboflavin, 0.5mg Niacin and 27mg Ascorbic acid.

Economic importance

Although labour and input requirements for French beans are higher than for maize, potato and wheat it's return per hectare per month is far much higher. An economic value can also be attached to the post harvest losses incurred. Lower post-harvest losses translate into lower marketing margin which benefits both consumers and producers.

French bear cultivation is a labour intensive exercise and thus it offers a means of livelihood to thousands of small scale farmers and also to people employed in large company farms. Production is a so semi-capital intensive and thus offers good business to the service industry especially the transport industry, banks and financial institutions where farmers seek funds to purchase seeds, chemicals, equipment and other farm

inputs.

Production of French beans in Less Developed Countries (LDCs) is estimated at 4 - 4.5 million tons. Demand by the year 2000 will be about 6.5 million tonnes. Projections in growth are based on approximations that the average population will grow by 1.3 - 2% while urban population growth rate will be 3 - 4 %. Over the same phase income growth will be 0.5 - 4.8 % and thus the income elasticity of demand will be 0.2 - 0.4 (Janssen *et al*,1992).

2.2 FRENCH BEAN PRODUCTION IN KENYA

The main export season for fresh produce is from October to May. Growers are advised to schedule their planting well such that the bulk of the produce is ready during the months of October to mid December, and from mid January to the end of May (HCDA, 1996). Production is carried out by both large and small scale farmers with the latter contributing a larger output. Cultivation is mainly along riverbeds and in irrigated areas of Embu, Meru and Mwea in Eastern province; Kiambu, Muranga and Nyeri in Central province; Naivasha and Nakuru in the Rift Valley; Bungoma, Trans Nzoia and Vihiga in the Western districts.

Presently Monel is the most widely grown variety. It is grown mainly for fresh export and to a lesser extent for canning. In 1987 trials on another variety called Finbel were done in various locations but it proved to be less preferred to Monel especially due to its poor establishment and greater pod length which posed problems in grading and packaging by the standardised methods for Monel (Njeru, 1989).

Newly introduced varieties include Claudia, Gloria, Maasai, Morgan, and Espanda (HCDA, 1996). Other varieties undergoing trial presently are Amy, Acardia, Celio, Duel, Garonel, Pure Gold, Rasada, Solido, Twiggy, Samantha, and Santara.

One major constraint in production has been the high input costs. French beans are vulnerable to attack by various arthropod pests and diseases amongst which rust has recently reached an alarming level. This coupled with poor post harvest and packaging technology, and a limited local market is reversing the trend of increasing production earlier reported by Mulandi (1988).

2.3 HARVESTING

Picking of pods begins six to eight weeks after planting depending on the area and continues for about one month. The pods are carefully picked and not pulled from the plants and must have the stalk attached to them. Picking should be at regular intervals ideally every other day in order to maintain export quality. Harvesting under rain or wet conditions is not recommended (HCDA, 1995; Njeru, 1989).

After harvesting sorting is done to remove broken, malformed and overgrown off types and insect damaged pods. Healthy pods are then graded into two main grades as defined by Kenya Bureau of Standards (KBS, 1983) specifications. Extra fine grade pods should be very tender, seedless w.th no strings and free from any defects. The maximum width (diameter) of the pods must be less than 6 mm and minimum length 10 cm. Fine grade pods may have small seeds and be short with soft strings. The width of the pods should be between 6 and 9 mm. In both grades the pods must

have the characteristic size and colour of its variety.

After grading the pods are packed in corrugated fibre board cartons or in plastic pre-packs. Before storage or transport pre-cooling is done using forced air coolers at 7 or 8°C. At 7 -8°C and 95 -100 % relative humidity the pods can be stored for one or two weeks. In Kenya beans for export are not usually stored for more than one day (HCDA, 1996).

2.4 BEAN RUST

The causal agent of bean rust is a basidiomycete of the order Uredinales, called *Uromyces appendiculatus* Pers; (Unger) [(Syn *Uromyces phaseoli* (Pers) G. Wint] (Robert, 1991). Its distribution is worldwide and its prevalent host is the common bean although it affects many other *Phaseolus* spp.and a few *Vigna* spp (Robert, 1991). It is of major importance on lima bean and on scarlet runner bean. The earliest reports of the pathogen were in 1797 (Westcott, 1950).

2.5 ECONOMIC IMPORTANCE OF BEAN RUST

Singh and Musyimi (1980) estimated a 37 % yield loss due to bean rust in Kenya. Heavy rust attacks can destroy a crop almost completely (Howland and MaCartney,1966). A rust severity range of 0.7-31.6 pustules cm^{-2} caused yield losses ranging from 336-1233 kg/ha which represented potential losses of 13-54 % (Venette and Jones, 1982). In 1981, a 54 % yield loss was recorded on snap beans and 78 % loss on dry beans (Baker *et al.*, 1985).

Yield losses can approach 100 % and are directly related to earliness of infection (Robert, 1991).In the U.S losses approach

a quarter of a billion dollars. Janssen and Guy (1992) estimated that fertilisers and pesticides constitute 13.53 % of the total production costs.

2.6 ETIOLOGY

The rust fungus is obligate and has an autoecious and macrocyclic life cycle. Basidiospore infection produces spermagonia (pycnia) on the adaxial surface of the leaf. Pycnia appear as chlorotic flecks that enlarge to about 2 mm in diameter and produce a white nectar containing the pycniospores. After movement of pycniospores to a pycnium of the opposite mating type cross fertilisation occurs and circular clusters (1-2 mm in diameter) of white aecia form on the abaxial surface of the leaf. Aecia produce colourless ellipsoid or oblong aeciospores that are 18-33 x 16-24 uM . Aeciospores infect beans to produce the amphigynous brown uredinia. Urediospores are 20-33 x 18-29 uM obovoid and broadly ellipsoid, cinnamon or golden brown and echinulate. Repeated generations of urediospore infections occur over most of the growing season.

Under appropriate conditions telia develop within aged uredinia and produce the chestnut brown (nearly black) ovoid or ellipsoid to globoid thick walled (with walls 2-4 uM) teliospores that are 24-35 x 20-29 uM. Immediately after the teliospores are formed, the two nuclei within the dikaryotic cells fuse to produce a large diploid nucleus. Following a period of dormancy teliospores germinate to produce a metabasidium in which meiosis occurs and on which are produced four binucleate or uninucleate basidiospores.

The basidiospores are reniform to ovate - elliptical, smooth hyaline 5.8-11.4 x 10.7 -20.uM. There are a few reports of teliospore germination and production of pycnia and aecia. Macrocyclic phase, however, is rarely found in nature (Westcott, 1950; Alexopoulos and Mims, 1979; Agrios, 1988; Robert, 1991).

2.7 EPIDEMIOLOGY

Infection by urediospores is favoured by moderate temperatures and duration of plant surface moisture for 10-18 hr (Robert, 1991). This is in agreement with the findings of 10 hr of moisture and temperature between 18 - 27°C required for infection (Bell and Daly, 1962; Shand and Shein, 1962; Imhoff *et al.*, 1981). At 15 - 22.5°C 90% germination occurred within the first 6 - 8 hr of wetness. Temperatures below 15°C retard uredinium development.

Prolonged temperatures greater than 32°C can kill the fungus (Robert, 1991). The latent period for the development of uredinial sporulation ranges from 7 days at the optimal temperature of 24°C to 9 days at 16°C. Abundant urediospore production is favoured by high humidity below the saturation point, long day length and young host tissue. Sporulation increased when plants were exposed to high humidity (Yarwood, 1961) while Cohen and Rotem (1970) reported that highest sporulation occurred following the longest photoperiod to which the host plants were exposed.

Spore release is greatest following a long dew period or rain. Efficiency of sporulation varies inversely with uredinium density. Urediospores are dispersed by water splashes to

neighbouring leaves and plants or can be dislodged and blown far away by wind and air currents (Howland and MaCartny, 1966; Hart and Saettler, 1981; Robert, 1991).

2.8 INFECTION

A rust spore on the host produces a germ tube which grows and develops an appresorium on contact with stoma (Wynn, 1976). An infection peg develops and grows between the guard cells until it reaches the substomatal chamber. A substomatal vesicle is formed which gathers the fungal cytoplasm and then elongates to form the infection hyphae. Haustoria form at the tip of the infection hyphae in contact with the host cell. The fungus penetrates leaf cells and proceed intercellularly throughout the host tissue eventually forming a young lesion. Host physiology and biochemistry are affected during the infection and sporulation process. A flow of labelled metabolites from the host cells to the haustoria, and then to the intercellular hyphae occurs. Hyphae, haustoria and spores of the fungus take up and accumulate metabolites from the host cells (Mendgen, 1979).

2.9 SYMPTOMATOLOGY

Rust affects leaves mainly but also pods and all other above ground plant parts (Robert, 1991). Symptoms begin as reddish brown, circular uredinial pustules on leaves or pods which raptures the epidermis to produce abundant, powdery urediospores. Pustules vary in size from pinpoint to 1 - 2 mm in diameter. Symptoms occur initially 5 - 6 days after infection as minute, whitish, slightly raised spots that enlarge slightly, rapture 7 -

9 days after infection to give reddish brown urediniospores.

After a few weeks no more urediospores appear but teliospores start forming (Agrios, 1988; Robert, 1991). Infected plants are predisposed to secondary infection by other organisms e.g. *Aschochyta fabae*. Severe rust results in defoliation, stunted growth and subsequent reduced yields. Pods infected shortly before harvest may be rejected in the market due to the development of disfiguring lesions.

2.10 SURVIVAL OF THE PATHOGEN

Urediospores (summer spores) eventually turn into teliospores (winter spores) which survive on bean debris and on poles used for staking the plant. After a resting period they are capable of germinating in the presence of moisture to produce basidiospores which cause infection in the next crop. Opio (1979) found that the possibility of seedborne inoculum causing infection was zero. She also did not find any infection from soil borne inoculum other than from infected plant debris. In tropical and subtropical regions, the fungus survives as urediospores surviving from season to season between crops growing at different times.

2.11 INOCULUM PRESERVATION

Howland and MaCartney (1966) reported that spores stored under low temperature had satisfactory viability but race reactions to the differentials had changed completely. While working on cowpea rust, Opio (1979) reported that the effect of a long storage period under varying temperature was manifested

in reduced germination. When stored at 2 - 4°C for 24 hr 76.5 % of the spores germinated. After 24 hr of storage the germination percentage decreased with increase of the storage period. The minimum time at which germination started was reported to increase with increase in storage period (Opio, 1979).

Kihurani (1989) did not report any reduction in the germination rate or host reaction differences after storing spores at -5-10°C. Spores remain viable for even two years if the leaves bearing them are dried for a few days at room temperature before storage at -20°C (Harter and Zaumeyer, 1941).

Germination is higher in spores from young leaves and pustules than from old leaves. Abundant infection can be obtained after spores have been stored in vials for over 600 days at -18°C provided that excess moisture is removed before storage. Under liquid nitrogen (-150°C), spores retain viability without loss of infectivity (Davison and Vaughan, 1963b; Cunningham, 1973). Stavely (1984) used spores stored at -18°C for differential host reactions.

2.12 RELATION OF HOST AGE AND LEAF POSITION ON PLANT SUSCEPTIBILITY

The relationship of the age of various host plants to disease susceptibility has been considered by several workers (Wyllie and Williams, 1965; Mence and Peg, 1971; Warren *et al.*, 1971; Dickson and Crute, 1974; Nyvall and Haughland, 1976; Hart and Endo, 1981; Njoya, 1991; Opio, 1979). Incubation period and susceptibility of cowpeas to rust caused by *Uromyces*

appendiculatus var vignae was largely affected by age of the plant (Opio, 1979).

Susceptibility of plants increased with age of the plants upto age of 18 days after which a decline was observed. Incubation was longer on older leaves than younger leaves. The most susceptible age of the leaf was 2 - 3 days, these leaves had the maximum severity regardless of the age of the plant.

Direct relationship has been reported between tobacco plants and blue mold *(Peronospora tabacina)* (Reuveni *et al.*,1986), lettuce infection by *Bremia lactucae* (Dickson and Crute, 1974) and the resistance of pea leaves to *Peronospora viciae* (Mence and Peg, 1971).

The age of plant and position of leaves on the stem influence the susceptibility of potato plants to *Phytophthora infestans* (Warren *et al.*, 1971) and also lower leaves of sunflower are more susceptible to infection by *Alternalia helianthi* than upper leaves (Allen *et al., 1983).*

2.13 TIME OF INFECTION AND THE GROWTH STAGE OF THE HOST

The degree of infection of plant or seeds maybe related to the time of infection as defined by the growth stage of the host . Bronniman (1968) related growth stages of wheat with infection by *Septoria nodorum.*

He found that infections occurring later had more effect than infections which occurred earlier. However, secondary infections occur at increasing maturation of the crop where early inoculation was made and direct infection of ears led to heavy infections except during the milk stage. Infection of Winter

wheat at the heading stage had a yield loss of 46 %.

A relationship has been established between the time of inoculation of winter wheat by *S.nodorum* and the amount of infection. The relationship is expressed as the number of conidia produced in the spikes and the corresponding reduction in the grain weight. Late inoculation after milky ripe stage had little or almost no effect, while early inoculation, when the ligule of the last leaf was just visible led to a reduction of the grain weight of about 40 %.

2.14 DISEASE CONTROL

Control by resistant cultivars

In 1935 Wingard had shown by histological studies that rust resistance in beans is a hypersensitive reaction. Stavely (1984) reports that CIAT efforts have led to considerable rust resistant germplasm being identified for both snap and dry beans. All genetic data on rust resistance in beans obtained indicate an oligogenic mode of inheritance but it has been theorised that considerable horizontal resistance might be available in already identified germplasm.

A problem that has frustrated breeding for resistance is the high potential for variability in the pathogen (Howland and MaCartney, 1966; Stavely, 1984). About 250 races of the pathogen have already been identified (Robert,1991). Kihurani (1989) reported the existence of many physiologic races of the pathogen in Kenya.

Chemical control

Farmers production strategy is based on a schedule of preventive chemical treatments. Juan and Pedro (1992) reported that if chemicals were not applied on time yields would be reduced by more than one third. Snap bean status as a cash crop makes it profitable to rely completely on chemical control (Ceasar and Pastor, 1992). Several chemicals have been tried with varying success.

The normal trend has been farmers depending heavily on application of cheap chemicals which incidentally happen to be less effective (Ken *et al*, 1987). Baycor, Bayleton and Plantvax have been tried and found effective (Bazirake, 1974; Opio, 1979; Singh and Musyimi, 1980; Ken *et al.*, 1987). Mancozeb, Blitox and other copper based fungicides did not give satisfactory control. Sulfur dusting was popular in the first half of this century (Westcott, 1950) but is no longer in use.

Although the use of chemicals is desirable as a control measure, various problems have arisen. Other than the prohibitive costs of fungicides, high applications have a dangerous effect on the environment and or human health. Blood samples from farm workers in Sumapaz, Columbia, showed significant levels of contamination (Janssen *et al.*, 1992). Juan and Pedro (1992) reported that over application, in addition to farmer toxicity leaves residues on the produce and also kills possible antagonists thus enhancing increase of the pests.

Mancozeb, a farmers 'avourite, sold as Dithane M 45 has been indicted worldwide for its notoriety in relation to residue levels on the produce. Ken *et al.* (1987), reported the rejection

of French bean produce from Florida, USA, by Canadian consumers due to high residue levels. Spraying the crop with an alternative fungicide especially the higher labelled rates of chrolothalonil is economically prohibitive to most farmers. Even mixing of fungicides has not helped to contain the high potential for new races in *Uromyces appendiculatus*. Success with chemicals as a management strategy, has only been partial, and therefore, alternative disease management methods must be sought.

Biological control

The antagonistic ability of *Bacillus subtilis* has been exploited in disease management. Stavely *et al.* (1981) reported the effectiveness of *Bacillus* dead cells to control bean rust in a greenhouse. Baker *et al.* (1985) reported on the evaluation of cultures and culture filtrates of the *Bacillus* sp in field control of bean rust. These applications gave good control but one application per week was not sufficient.

Baker *et al.* (1983) reported their findings on several antagonists, some of which gave good control ranging from 86 to 99 %. Amongst them, the most promising were two *Bacillus subtilis* isolates, *B.cereus* subsp *mycoides*, *B.thuringiensis* and *Erwinia ananas* pv *uredovora*. The same workers reported on the phytotoxic effect of one *Bacillus* isolate products on the crop. This phytotoxicity resulted in the crops being much greener and more succulent but with a marked decrease in yield (Baker *et al.*, 1984). The antagonistic capacity of *Bacillus* sp has been reported on *Alternalia alternata* (Deborah and Harvey, 1977), *Helminthosporium sativum* and *Macrophomina phaseolina*

(Thirumalacar and O'Brien, 1977). Spencer (1980) reported on the activity of *Verticillium lecanii* against *Uromyces appendiculatus*. From the foregoing it is clear that potential for biological control exists.

Cultural control

Crop rotations with non hosts such as maize has been suggested but there are no reports to show how successful it can be (Robert, 1991). Sanitation by burning and burying of crop debris has also been cited as being successful. Disinfection of posts and even tools between fields is also suggested as a supplement to other efforts. Adjustments of planting dates and also reduction of plant density in the fields have been suggested as possible control measures (Robert, 1991).

2.15 INTEGRATED PEST MANAGEMENT (IPM)

There is overwhelming evidence that pesticides alone do not lead to sustainable pest management in agriculture. The cost of the chemicals and their long term deleterious effect on the environment and health argue against the continued prodigious use of pesticides inspite of earlier spectacular success (Kibata, 1989). Pest management practices should recognise all components of the inherently fragile agricultural ecosystem.

IPM requires the farmer to be knowledgable about the identity and role of beneficial insects and other biological control agents, the role and potential disadvantages of pesticides use and abuse, and a wide array of cultural and crop sanitation practices that reduce pest incidence (Anonymous,

1989). Little IPM has been tried for snap beans (Ceaser and Pastor, 1992). For a rational IPM programme in French beans, especially for control of bean rust the following factors should be considered:

(a) A more rational chemical control as one of the components of IPM in addition to a broad genetic base, use of resistant varieties, use of clean seed or seed treatment and crop rotations.

(b) Establishment of the economic thresholds and the critical periods of control.

CHAPTER 3:MATERIALS AND METHODS

3.1 FIELD SITES

This study was conducted at Kabete Field Station, Faculty of Agriculture at University of Nairobi and at Homegrown (K) Ltd Marula farm, Naivasha.

Kabete Site

The Kabete station is about 1940 m above sea level and it lies within latitudes 1^0 14 '20'' to 1^0 15' 15'' S and longitudes 36^0 44' to 36^0 45' 20'' E (Wamburi, 1973). It has a bimodally distributed rainfall with the long rains starting from late March to June and short rains from late October to December. The mean annual rainfall of the station is 925 mm and the potential evapotranspiration is 1363 mm. The soils at the experiment site are deep red entric nitosols containing 60 % clay particles. The ay mineral is predominantly kaolin while the parent material the Kabete trachyte. The pH of the soil ranges between 5.2 - 2 for the top soil and 5.2 and 7.7 for the subsoil (Nyadat and chieka, 1970).

Naivasha Site

The Naivasha farm is located in the region of longitude latitude co -ordinates 36^0 25' to 36^0 50' E and 0^0 37 to 0^0 54' S. It lies about 2600 m above sea level. The climate is warm and semi-arid. The zone experiences a double rain shadow effect East and West from the flanking escarpments and as a result the basin receives less rainfall than the surrounding highlands. Average rainfall is about 665 mm p.a. The rainfall has a bimodal

distribution with the main pulse in April and May and a minor pulse in November.

The amount of rainfall is low and evaporation higher, and therefore the moisture is not enough for rain fed agriculture but with irrigation the area has good potential for vegetable production (Ralph and Helmut, 1983). Air temperatures are moderate with a monthly mean varying little from 15.9 to 18.5° C. Light breezes are common in the morning but stronger afternoon winds (11 - 15 km/h) are typical.

The farm lies on volcanic plains of slope 0 -5 %, and it has fine sandy to sandy loam or silt soils classified as Ando calcaric andosols. These soils have moderate to high fertility (NES, 1994).

3.2 FIELD SURVEY

Farmers fields in the following four French bean growing districts were visited :

1. Mwea division (Embu district).
2. National Horticultural Research Centre (Thika district).
3. Homegrown Marula farm (Naivasha)
4. Kabete University farm (Nairobi).

The objective was to collect samples of urediospores from different environments and climatic conditions. A homogenized sample representative of a number of rust races was assembled by mixing and multiplying the collected spore samples. Spore collection was done continuously between December 12, 1995 and January 24, 1996.

3.2.1 Spore collection and preservation

Mature urediospores were collected by tapping leaves bearing pustules over flimsy paper. Sieve No.200 was used to separate spores from debris. Purified spore samples were put in dry vials which were then plugged with cotton wool and put in a desiccator at room temperature (23 ± 2 °C) for 2 - 3 days as recommended by Kihurani(1989). The dried spores were then stored in tightly closed vials at 4 ^{0} C.

3.2.2 Spore multiplication

Samples of the purified spores from different locations were mixed in about equal proportions for multiplication. Multiplication was done on pre-germinated bean plants of cv GLP [illegible]ch is known to be very susceptible to bean rust (Kihurani,

Five cv GLP 2 bean seeds were planted in 15 cm diameter [illegible] in the greenhouse and thinning was done to three uniform [illegible] per pot after germination (5 - 7 days). The composition [illegible] pot mix was 2: 1: 1 - soil, sand, manure (v/v). Twenty five grams of Di-Ammonium Phophate fertilizer were added per 20 litre volume of the mixture.

The mixture was sterilized at 121^{0} C and 6.89 Kpa pressure for 15 minutes and allowed to stand for four weeks before use so as to allow any gases released during sterilization with possible phytotoxic effects to diffuse off. Plants in the greenhouse were watered manually on alternate days. At the third trifoliate leaf stage the plants were top-dressed with Calcium Ammonium Nitrate at a rate of 5 gm per 10 pots.

Inoculation of the plants

Plants were inoculated when the primary leaves were well expanded (10-12 days after planting). Inoculum was made using urediospores earlier harvested and preserved as described in Sec 3.2.1. The urediospores were put in sterile distilled water containing a few drops of Tween 80 for effective dispersal. Concentration was adjusted to 2×10^4 spores/ml using a haemocytometer. Two alternative methods of inoculating beans were initially used.

In method one a camel hair brush was used to apply the dry urediospores onto the upper surface of leaves. Only few pustules developed and so this method was discarded. In the second method which was more preferred inoculation was achieved by applying a fine spray of the inoculum onto the upper surface of the leaves from a distance of 30 cm until runoff. The hand sprayer used was a Canyon model 5A.

Inoculated plants were covered with transparent polybags to increase humidity at room temperature (23 ± 2^0 C) for 24 hr. A new generation of urediospores was ready for harvesting 8 - 10 days after inoculation. Harvesting and preservation was done as described in Sec.3.2.1.

3.3 EVALUATION OF *Bacillus* sp AS BIOLOGICAL CONTROL AGENTS

Two isolates of the *Bacillus* sp coded CA 1 and CA 5 were obtained from Dr.E.W. Mutitu of the Department of Crop Science University of Nairobi. The bacteria had been preserved in sterile modified loam soil. The bacteria were retrieved by sprinkling particles of the carrier onto the surface of Nutrient Agar (NA)

in a sterile environment.

Colonies formed on the inoculated plates after incubating for periods of 24 - 36 hr at room temperature (23 ± 2^0 C).Pure cultures of each isolate were obtained by inoculating fresh NA plates with cells from single colonies formed on the old plates. Inoculation was done by streaking the surface of NA using a sterile loop wire with the bacterial cells. The inoculated plates were incubated for 24 hr at room temperature (23 ± 2^0 C) for colonies to develop.

3.3.1 Growth of bacterial cultures for evaluation

A bacterial suspension of *Bacillus* sp was prepared by flooding a Nutrient Agar plate of pure colonies of each isolate with 10 ml of sterile distilled water and scraping the surface using a sterile glass rod to dislodge the bacterial cells. The prepared suspension was transferred to one litre flasks of Nutrient Broth.

For each *Bacillus* sp the Nutrient Broth was inoculated with the suspension at a ratio of 5 :100,inoculum : media (v/v). Inoculated medium was incubated on a circulatory shaker at 125 r.p.m at room temperature (23 ± 2^0 C) in the dark as recommended by Baker *et al* (1983; 1985).Darkness was simulated by wrapping the growth flasks with aluminium foil.

Starting from the third day 5 ml samples of each isolate were drawn from the growth flasks using a sterile pipette and preserved in closed vials at 2 - 3^0 C for use in a spore germination bioassay. Culture growth was stopped after seven days of growth on the shaker.

3.3.2 Spore germination bioassay

Dry urediospores were put in water containing Tween 80 to aid in spore dispersal.Urediospores of the bean rust fungus contain methyl -cis - 3, 4 dimethoxycinnamate (MDC), a cinnamic acid ester which inhibits spore germination (Wolf,1982). This inhibitor was removed by leaching the spores with water. The spores were made to float on water for periods of 10 minutes after which the water was sucked out using a pasteur pipette. This procedure was repeated four times. The spores were then re-suspended in sterile distilled water.

A drop of the spore suspension was placed on a cavity slide where one drop of the bacterial suspension had been put earlier. By doing this a 50 % dilution of the spore suspension and of the bacterial suspension was accomplished. Bacterial samples drawn from the third to the sixth day were used for the spore germination test. The cavity slides were placed in a completely randomised manner on moist blotter papers in a baking dish and covered at 18^0 C for 24 hr in an incubator.

Water which had been heated to boiling point to mobilize inorganic nutrients and then cooled to room temperature (23 ± 2^0 C) was included as a control germination medium as recommended by Baker *et al* (1983,1985). Each treatment was replicated four times.

3.3.2.1 Data recording and analysis

After 24 hr the slides were observed under a microscope and the number of germinated spores was recorded.

Analysis of Variance (ANOVA) was done. Separation of means was

done using the Duncan's Multiple Range Test.

3.3.3 Determination of the growth rate of *Bacillus* sp during a seven day incubation period at room temperature

Samples of the two bacterial isolates CA 1 and CA 5 were drawn from the growth media starting from the third day of incubation to the seventh day as described in Sec 3.3.1 . Each isolate was serially diluted up to 10^{-10} using sterile distilled water.

One millilitre of culture broth was transferred to 9 ml of the water blank and the mixture shaken thoroughly to give 10^{-1} dilution. From this dilution 1 ml was transferred to another 9 ml of the water blank and shaken well to give 10^{-2} dilution. The procedure was repeated up to the 10^{-10} dilution.

For each isolate inoculation of the NA plates was done by transferring 0.1 ml of each of the last five dilutions onto the surface of the medium using a sterile pipette. The bacterial suspension was spread evenly on the surface of the medium using a bent sterile glass rod. For each dilution three replicate plates were made. The inoculated plates were incubated at room temperature (23 $\pm$ 2^0 C) on the laboratory bench for colonies to form.

Colony counting

After 48 hr the colonies formed were counted and the average taken for each dilution level. The data obtained were used to calculate the concentration of colony forming units per millilitre (cfu/ml) of the original suspension.

For each dilution level used the formula used for calculation was : CFU/ml = colony count X dilution factor X 10. Log_{10} of cfu/ml for different incubation periods were used to plot comparable growth curves of the two *Bacillus* sp isolates.

3.3.4 Effect of bacterial cell suspensions against bean rust in the greenhouse

For each isolate bacterial cultures harvested after incubating for seven days were diluted with an equal amount of sterile distilled water to make a suspension. For each bacterial isolate half of the culture broth was autoclaved for 15 minutes at 121 ^{0} C and 6.89 Kpa pressure while the other half was not. Bean plants of cv. Monel were raised in the greenhouse as described in Sec.3.2.2 and were ready for use when their primary leaves were well expanded (10 - 12 days after planting). Plants were first sprayed with the bacterial suspension prepared above using a Canyon model 5A hand sprayer from a distance of 30 cm so as to fully cover both sides of the leaves.

Inoculum preparation and application

A spore solution was prepared as described in Sec 3.2.3. When the bacterial suspension applied on the leaves had dried the plants were sprayed with the spore solution described in Sec 3.2.3. The control plants were sprayed with distilled water before applying the rust spores. Plants sprayed with Dithane M-45 (Mancozeb) at a rate of 2.5 gm /l before inoculation with the rust spores were used as a standard check. Six treatments were prepared as follows:

(a) Water.

(b) Mancozeb.

(c) Isolate CA 1 autoclaved suspension.

(d) Isolate CA 5 autoclaved suspension.

(e) Isolate CA 1 live cell suspension.

(f) Isolate CA 5 live cell suspension.

Each treatment was replicated three times. Inoculation was done as described in Sec 3.2.3. The treated plants were covered with polythene bags and incubated for 24 hr at room temperature (23 ± 2^0 C). The plants were assessed after 8 days for rust infection. Out of the three plants in a pot the one in which infection on the two primary leaves was most similar was selected and the rust pustules formed on it counted. Six leaves were therefore considered per treatment. Data recorded was analyzed as described in Sec 3.3.2.1.

3.4 EVALUATION OF SEED TREATMENT WITH SYSTEMIC FUNGICIDES

Six fungicides were evaluated for systemic activity against the bean rust fungus when used as seed dressings. The fungicides were Anvil (Hexaconazole), Baycor (Bitertanol), Saprol (Triforine), Raxil 025 (Tebuconazole), Raxil 040 (Tebuconazole + Triazoxide) and Real (Triticonazole). The first three are regularly used as systemic foliar fungicides against rust while the other three were undergoing their first trials on bean rust. Each of the fungicides was evaluated at three levels of concentration in the laboratory and in the greenhouse.

Table 1: Chemical fungicides and the rates evaluated

Trade name:	Chemical name :	Rates Low	Medium	High
Anvil	Hexaconazole	0.1ml/100ml	0.2ml/100ml	0.3ml/100ml
Baycor	Bitertanol	0.1ml/100ml	0.2ml/100ml	0.3ml/100ml
Saprol	Triforine	0.1ml/100ml	0.2ml/100ml	0.3ml/100ml
Rakil 040	Tebuconazole Triazoxide	0.4ml/0.5kg	0.75ml/0.5kg	1.1ml/0.5kg
Rakil 025	Tebuconazole	0.4ml/0.5kg	0.75ml/0.5kg	1.1ml/0.5kg
Real	Triticonazole	0.625ml/0.5kg	1.25ml/0.5kg	1.875/.5kg

3.4.1 Preparation of the chemical solutions and application on the seeds.

Anvil, Baycor and Saprol are formulated as liquids and were prepared by taking an appropriate volume of each chemical and mixing it with water to get the proper concentration.
Real, Rakil 040 and Rakil 025 are formulated as flowable solutions suitable for seed dressing. A desirable volume of each were diluted with water at a ratio of 1 : 1.5, chemical : water (v/v).

Seeds of Monel bean variety were used throughout. For the liquid formulations (Anvil, Baycor and Saprol) seed treatment was by the steep method. Half kilogramme quantities of seed were soaked in the chemical solutions for 15 min after which they were drained and air dried by spreading on a sheet of paper (Neergaard, 1976).

For flowable solutions half kilogramme quantities of seed were put in glass containers. The chemical was poured so as to

wet the sides of the container and ensure uniform distribution. Seeds were shaken in the closed container to ensure uniform coating of the seed surface by the chemical. Regular shaking of the containers to turn the treated seeds while exposed to air enhanced their drying.

3.4.2 Testing the effect of storage on seed germination after seed treatment

The effect of fungicides on seed germination was evaluated 24 hr and two weeks after seed treatment. Three hundred seeds per treated sample were taken and a germination test conducted. One hundred seeds were rolled in moistened blotter papers and incubated in a completely randomised manner in a humidity chamber at room temperature (23 ± 2^0 C) for seven days. The humidity chamber structure was made of wire frame and the sides covered with polythene sheets.

The seeds rolled in blotter papers were moistened daily by sprinkling with water. An untreated seed sample was included at both evaluation periods as a control. The treatments were replicated three times.

After seven days the number of germinated seeds per replicate of each treatment was recorded. Data analysis was done as described in Sec 3.3.2.1.

3.4.3 The effect of seed treatment on delaying rust infection in the greenhouse.

Five French bean seeds of each treatment were raised in the green house as described in Sec 3.2.2.

Inoculum preparation and application

Inoculum prepared as described in Sec 3.2.3 was initially sprayed onto the upper surface of well expanded primary leaves of the bean from a distance of 30 cm until runoff. Inoculation was carried out in a wood frame chamber measuring 4.5 m long x 1.5 m wide x 0.6 m high with sides covered by medium gauge transparent polythene sheets.

After inoculation plants remained in the chamber at room temperature (23 ± 2^0 C) for 24 hr.

The experiment had the following treatments :

(a) Six chemicals each at three concentration levels.

(b) One untreated seed sample as control.

Data recording and analysis

Observations for infection started seven days after inoculation and the following records were taken:

(a) Delay in infection was recorded as the number of days from day zero to when 50 % of the plants in a plot (three replicate pots,each pot having three replicate plants) were infected. Day zero was considered as the day when the first inoculation was done.

(b) Incidence data were recorded as the number of leaves infected per plant and as the number of infected plants per plot.

(c) Severity data was recorded as the percentage leaf area covered by pustules estimated using the modified Cobb scale (Fig 1) (Aart and Martial,1987).

Data analysis was done as described in Sec 3.3.2.1.For each treatment the severity data recorded were used to calculate rates

of disease increase and to plot disease progress curves.

3.5 EVALUATING THE EFFECT OF SEED TREATMENT IN THE FIELD

Seeds were treated with six fungicides as follows:

(a)	Anvil	0.2 ml	/100ml water
(b)	Baycor	0.2 ml	/300ml water
(c)	Saprol	0.2 ml	/100ml water
(d)	Real	1.25 ml	/0.5 kg seed
(e)	Rakil 025	0.75 ml	/0.5 kg seed
(f)	Rakil 040	0.75 ml	/0.5 kg seed

The seeds were planted 24 hours after treatment.

3.5.1 Experimental design

Kabete site

The experiment was laid out as a Randomised Complete Blocks Design (RCBD) with three blocks. Each block measured 4 m x 28.5 m and had 8 plots. Each plot measured 3 m x 4 m and plots had paths of 0.5 m between. Spacing was 30 cm x 10 cm between and within rows respectively. Di-Ammonium Phosphate fertilizer was applied at a rate of 200 kg/ha at planting.

In each plot a spreader row of cv GLP 2 seed, known to be highly susceptible to rust was planted after every four rows of French beans. Guard rows had been planted two weeks before the main experiment and artificially inoculated with rust.

Naivasha site

Land preparation was mechanized and planting was done in plots measuring 3 beds by 3 m long each. Each bed had four rows

with a distance of 45 cm between them. Spacing was 45 cm x 8 cm between and within rows respectively. The experimental design and layout was as for the Kabete site.

The eight treatments included were:

(a) Six seed samples each treated with a different chemical.

(b) One untreated seed sample where disease was not controlled.

(c) One untreated seed sample where disease was controlled throughout the growing season by weekly foliar applications of Mancozeb at a rate of 2.5 gm /litre of water.

3.5.2 Inoculation

Inoculum was prepared as described in Sec 3.2.3 and spore concentration was adjusted to 2×10^6 spores/ml. This inoculum was sprayed onto the trial plots using an Osatu backpack knapsack at a rate of 1000 l / ha. The initial inoculation was done when more than 50 % of plants in the treatment plots had attained the primary leaf stage.

Data recording and analysis

Disease severity and incidence data were recorded weekly for eight weeks starting seven days from the date of initial inoculation until the end of the harvesting period. For all the treatments 30 plants per plot were randomly selected and tagged. Data were observed from the tagged plants throughout the duration of the experiment.

Harvesting started when more than 12 % of the pods formed were on average 6 mm diameter and 10 cm long (KBS 1983). The pods were carefully picked and not pulled from the plants and had the

stalks attached to them. Picking at Kabete was done on alternate days while at Naivasha it was done daily. Pods picked were graded into either fine or reject grades as described in Sec 2.3. Different grades were weighed separately .

The disease severity data obtained was used to calculate the rates of disease increase and to plot disease progress curves. Area under the disease progress curves (AUDPC) was calculated using the formula given by Shaner and Finney (1977).The formula is $AUDPC = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2][X_{i+1} - X_i]$

Where i =1, 2,....,n

n =total number of observations

Y_i =Severity score recorded at the i^{th} observation

X_i =Number of days at which observation is done

Differences in values of AUDPC were analyzed for significance and separation of means was done using the Duncans Multiple Range Test.Yield analysis was done on the total weight of the yield obtained as described in Sec 3.3.2.1.

3.6 EVALUATION OF THE EFFECT OF THE STAGE OF INFECTION ON YIELDS.

Out of the nine stages of development of a bean plant six were considered for evaluation. The stages considered were :

(a) V 2 ..... primary leaves

(b) V 3 ..... first trifoliate leaf

(c) V 4 ..... third trifoliate leaf

(d) R 5 ..... pre-flowering

(e) R 6 ..... flowering

(f) R 7 ..... pod formation.

where V is for those stages that are in the vegetative phase while R is for reproductive phase.

3.6.1 Experimental design

Kabete site

The experiment was laid as a Randomised Complete Blocks Design (RCBD) with three blocks. Each block had seven plots. Plot sizes and planting distances were as described in Sec 3.5.1.

Naivasha site

The experiment was laid as a RCBD with three blocks. Each block had seven plots.Plot sizes and planting distances were as described in Sec 3.5.1.

Treatments included were the six stages of growth and one plot kept disease free by weekly foliar applications of Mancozeb at the rate of 2.5 gm / lit water.

3.6.2 Inoculation

Inoculum was prepared as described in Sec 3.2.3. Inoculum was applied when 50 % of the plants attained the characteristics typical of the infection stage being treated, for example for stage V 2 inoculation was done when 50 % of the plants had their primary leaves unfolded. Inoculum was sprayed onto the upper leaf surfaces using an Osatu backpack knapsack at an application rate of about 1000 lit /ha.

At Kabete where the disease was not prevalent cv GLP 2 plants grown and inoculated with rust in the greenhouse were placed strategically next to the treatment plants.

Plots whose stage of inoculation was not due were kept rust free by weekly foliar applications of Mancozeb at a rate of 2.5 gm /lit water. This fungicide has only protectant activity and was easily washed off by irrigation water within a week and so plots requiring inoculation could be treated with minimum interference from chemical residues on the leaf surface.
Data on disease severity, incidence and yield were recorded and analyzed as described in Sec 3.5.3.

3.7 COMPARING THE EFFECT OF EARLY AND LATE FOLIAR SPRAYS OF THREE FUNGICIDES ON DISEASE DEVELOPMENT AND YIELD.

Results from Expt 3.6 indicated that infection of the bean plant at certain stages of development was critical and affected yield. This experiment was designed to determine whether protecting a bean crop with systemic fungicides from the time of rust infection would be of any advantages over chemicals applied later when the plant reaches the pre-flowering stage. Pre-flowering stage was shown to be the one at which rust infection depressed yields most.The three fungicides used in the trial are commonly used as foliar sprays with systemic activity against bean rust. These were Baycor (Bitertanol), Anvil (Hexaconazole) and Saprol (Triforine). Cell suspensions of two *Bacillus* sp isolates were included for evaluation of their effect on bean rust under field conditions at the Naivasha site.

3.7.1 Experimental design

Kabete site

The experiment was laid as a Completely Randomised Block Design with 21 plots. Plot sizes and planting distances were as described in Sec 3.5.1.

The treatments included were:

(a) Three plots which were sprayed each with Anvil, Baycor or Saprol starting as soon as disease was spotted after opening of the primary leaves.

(b) Three plots which were sprayed each with Anvil, Baycor or Saprol starting at the third trifoliate leaf regardless of whether infection had set earlier.

(c) A control plot which was not protected against rust infection throughout the season.

Each treatment was replicated thrice.

Naivasha site

In addition to seven treatments similar to those at the Kabete site, cell suspensions of two *Bacillus* sp isolates were included making a total of nine treatments. The experiment was laid out as a Completely Randomised Block Design with 27 plots. Plot sizes were as described in Sec 3.5.1.

3.7.2 Application of treatments

(a) Bacterial cell suspensions

For each of the *Bacillus* sp isolates the bacterial cells were grown as described in Sec 3.3.1 for seven days. The culture broth was then autoclaved to inactivate the bacterial cells. The

spray solution was prepared by diluting the autoclaved culture broth with an equal amount of water. The bacterial suspension was sprayed on the plants as soon as the primary leaves had opened before the onset of rust. Weekly applications were carried out. Plants were sprayed using an Osatu backpack knapsack sprayer so as to ensure thorough coverage of all above ground parts.

(b) Chemical sprays

For each fungicide the treatments started as soon as infection set in after opening of the primary leaves on one set of the treatment plants. The second set of treatments started when the beans were between the third trifoliate leaf and the pre-flowering phase. Weekly applications were carried out. For all treatments data on disease severity, incidence and yield were recorded and analysed as described in Sec 3.5.3.

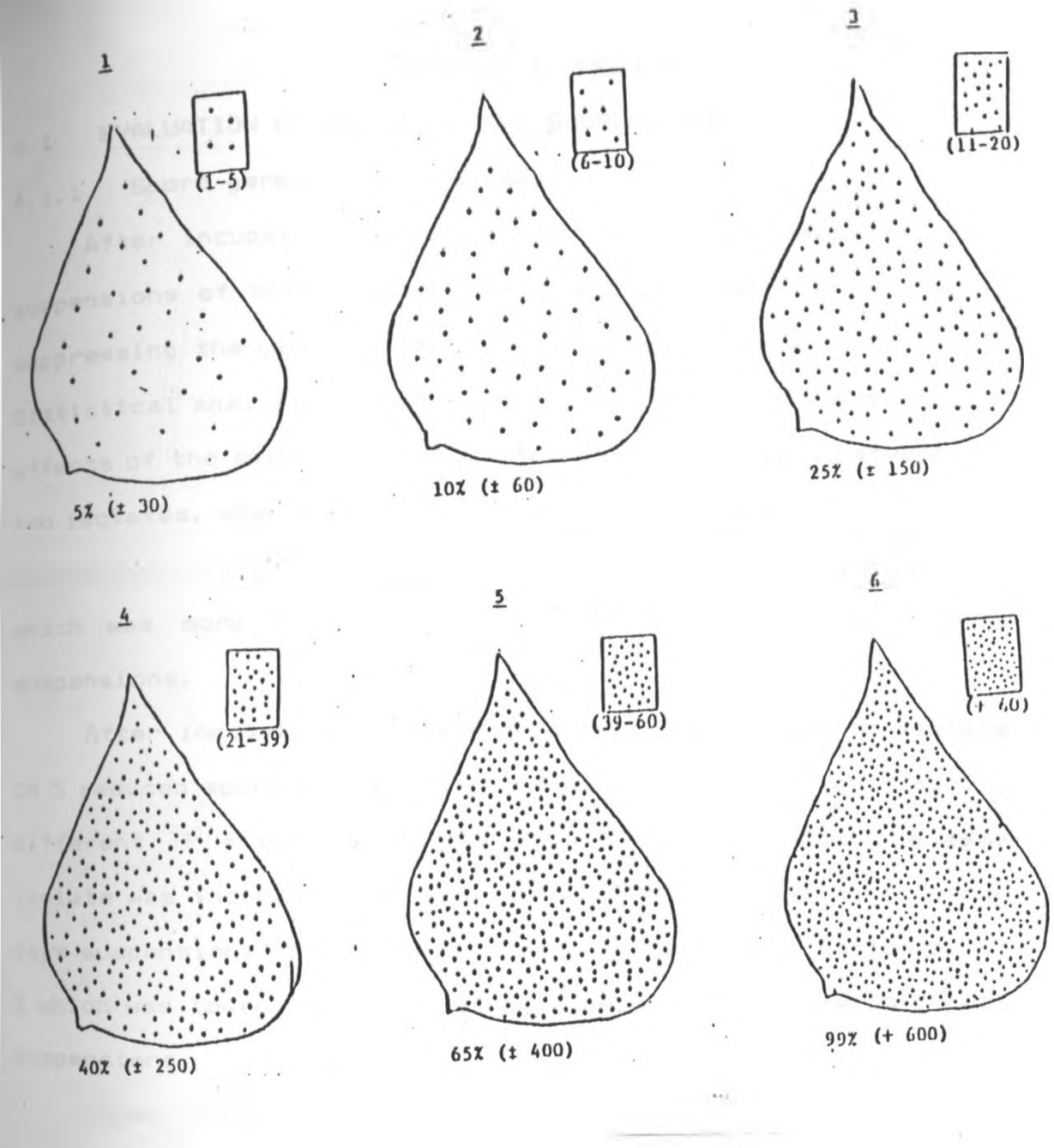


Fig 1: The Modified Cobb Scale for estimating bean rust intensity. The diagram shows six degrees of rustiness which were used in estimating the percentage of rust infection on the leaf. The shaded spots represent rust, and the figures represent approximately the rust percentage computed on the basis of the maximum amount of surface covered by rust as shown in the 99 % figure. Thus figure No.6 represents 37 % of actual surface and is arbitrarily selected as 99 %. Other percentages are in terms of No.6.

CHAPTER 4: RESULTS

4.1 EVALUATION OF BACILLUS CELL SUSPENSIONS

4.1.1 Spore germination bioassay

After incubating for more than three days bacterial cell suspensions of both isolates CA 1 and CA 5 were effective in suppressing the germination of urediospores (Plate 1, Table 2). Statistical analysis showed significant differences between the effects of the suspensions at different ages and also between the two isolates. When assessed after incubating for three days, cell suspensions of isolate CA 5 reduced spore germination by 80.4 % which was more than the 68.2 % recorded for isolate CA 1 suspensions.

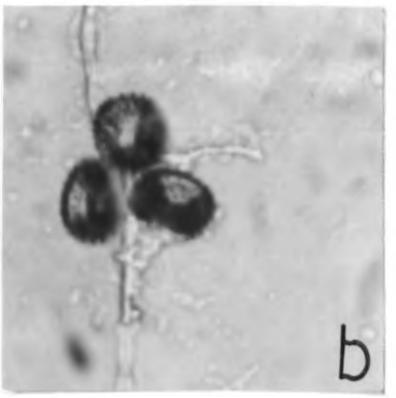
After incubating for four days cell suspensions of isolate CA 5 reduced spore germination by 90.2 %. This was significantly different ($P = 0.05$) from the 80.4 % recorded when the same isolate was incubated for three days. After incubating for four days suspensions of isolate CA 1 reduced spore germination by 80 % which was lower than the 90.2 % recorded for isolate CA 5 cell suspensions.

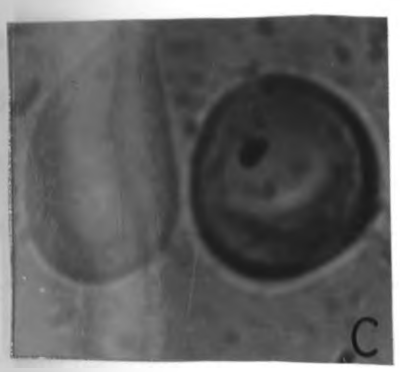
After incubating for five days suspensions of isolate CA 5 reduced spore germination by 97.5 % while isolate CA 1 suspensions reduced spore germination by 92 %. After six days in incubation isolate CA 5 suspensions completely inhibited spore germination while isolate CA 1 reduced spore germination by 98.7 %. Isolate CA 5 cultures were more effective in suppressing spore germination than isolate CA 1 cultures.

Table 2: Effect of *Bacillus* sp Isolate CA 1 and CA 5 cell suspensions incubated for periods of 3 - 7 days in Nutrient Broth on the germination of urediospores in the laboratory.

	CA 5		CA 1	
Treatment	Germinated spores (mean)	% reduction	Germinated spores(mean)	% reduction
Heated water	41.0 a		45.0 a	
3 day culture	8.0 b	80.4	14.3 b	68.2
4 day	4.0 c	90.2	9.0 c	80.0
5 day	1.0 d	97.5	3.6 d	92.0
6 day	0.0 d	100.0	0.6 e	98.7

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncans Multiple Range test (P =0.05).





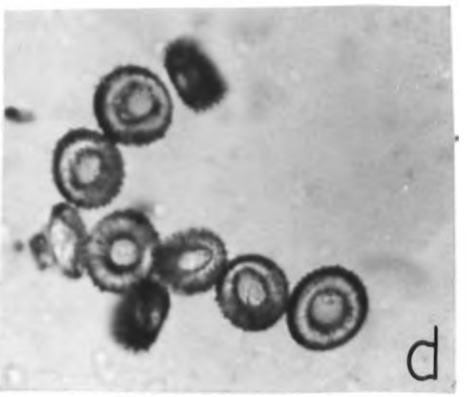


Plate 1
Effect of different types of *Bacillus* cell suspensions (in media) on the germination of urediospores in the laboratory.
(a) Well germinated urediospores in water heated to boiling point then cooled to room temperature.
(b) Spores germinated in 3 day old isolate CA1 cell suspension.
(c) + (d) No germination in 5 day old suspensions of isolate CA1 and CA5 respectively.

4.1.2 Determination of concentration of bacterial cells in suspensions incubated for periods of 3 - 7 days in Nutrient Broth

Results showed that isolate CA 5 formed colonies within 24 hr after plating on Nutrient Agar, while CA 1 needed 36 hr for distinct colonies to form. For both isolates the rate of increase was highest between the fifth and the sixth day of incubation. at each dilution level cell suspensions of isolate CA 5 had a higher viable cell count than those of isolate CA 1 (Table 3). After incubating for three days suspensions diluted to 10^{-6} had 464 and 924 colony forming units (cfu/ml) for isolate CA 1 and CA 5 respectively. Suspensions diluted to 10^{-10} had 2 and 34 cfu/ml for isolate CA 1 and CA 5 respectively. After incubating for four days isolate CA 1 had 3.5×10^{10} cfu/ml while CA 5 had 4.44×10^{11} cfu/ml. After incubating for seven days isolate CA 1 had 1.54×10^{12} cfu/ml while isolate CA 5 had 8.16×10^{12} cfu/ml (Table 3; Fig 2).

Table 3: Concentration of cells in suspensions of *Bacillus* sp isolate CA 1 and CA 5 after incubating for periods of 3 – 7 days on a shaker in the laboratory.

Incubation period	Isolate CA 1		Isolate CA 5	
	cfu/ml	$\log_{10}$(cfu)	cfu/ml	$\log_{10}$ (cfu)
3 days	1.09^{10}	10.0	1.03^{11}	11.0
4 days	3.5^{10}	10.5	4.44^{11}	11.6
5 days	1.72^{11}	11.3	1.93^{12}	12.3
6 days	9.44^{11}	11.9	8.16^{12}	12.9
7 day	1.54^{12}	12.2	Too many to count	

cfu/ml is the number of colony forming units per millilitre of the original suspension.

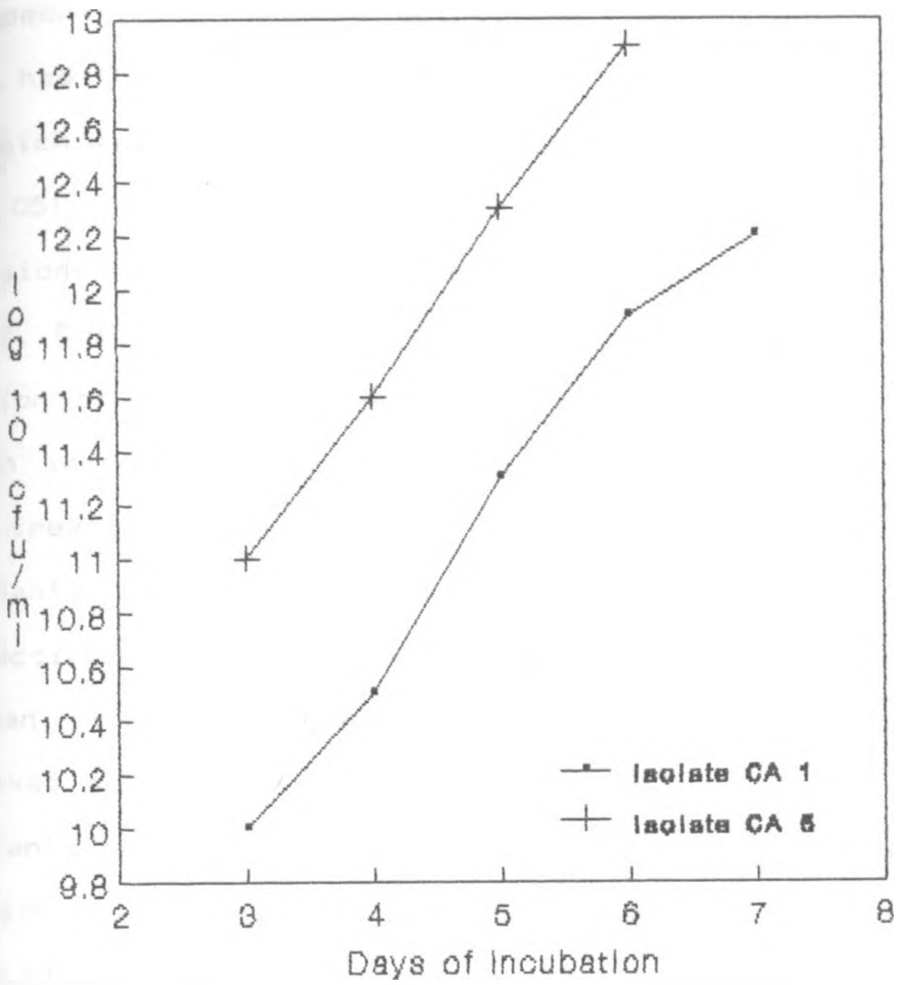


Fig 2: Growth curves of Bacillus sp isolate CA1 $ CA5 after incubation for 7 days in Nutrient Broth.

4.1.3 Greenhouse assessment of bacterial cell suspensions

Live cell suspensions of both isolates suppressed rust development more than the autoclaved suspensions. The control plants had the highest infection with a mean of 15 pustules per leaf which was significantly different from all other treatments ($P = 0.05$) (Table 4). Plants treated with isolate CA 5 live cell suspensions had the least infection with a mean of 0.5 pustules perleaf. Compared to the highest infection this represented a reduction of 97.3 % and was significantly different ($P = 0.05$) from an infection mean of 2.8 pustules per leaf observed on plants treated with autoclaved suspensions of the same isolate.

Plants treated with isolate CA 1 live cell suspensions had an infection mean of 2.3 pustules per leaf. This was less than the mean of 4.7 pustules per leaf on plants treated with autoclaved suspensions of the same isolate.

Plants treated with Mancozeb had a mean of 1.5 pustules per leaf which was a significantly higher score than 0.5 pustules per leaf on plants treated with isolate CA 5 live cell suspensions. Autoclaving reduced the effect of isolate CA 5 and CA 1 by 16 and 15.7 % respectively.

Table 4: Effect of cell suspensions of *Bacillus* sp isolates CA 1 and CA 5 on development of rust pustules on French beans in the greenhouse at Kabete.

Treatment	Pustuales per leaf (mean)		% reduction
Water	15.0	a	--
CA 1 Autoclaved	4.7	b	68
CA 5 Autoclaved	2.8	bc	81.3
CA 1 live cell	2.3	bc	84.7
Mancozeb	1.5	bc	89.3
CA 5 live cell	0.5	c	97.3
Mean	4.5		
S.E	1.1		

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncans Multiple Range Test (P =0.05).

Cell suspensions had a delaying effect on the latent period of infection. Pustules developed earliest on the control plants after eight days while they developed after twelve days on the plants treated with isolate CA 5 live cell suspensions. On the plants treated with isolate CA 1 live cell suspensions rust pustules were visible between the tenth and eleventh day after inoculation. This was similar to the pustules observed on plants treated with Mancozeb and those treated with isolate CA 5 autoclaved suspensions.

Plants which were treated with live cell suspensions of both *Bacillus* sp isolates exhibited phytotoxic effects. On the bean plants the leaf margins of treated plants were scorched and the apex acquired a round shape different from the normal angular pointed shape (Plate 2). Their leaves were curled with dark green patches in the central regions of the lamina giving them a malformed appearance. The phytotoxic effect was more enhanced in plants treated with isolate CA 5 live cell suspensions than on those treated with CA live cell suspensions. Plants treated with autoclaved suspensions of both isolates did not exhibit the scorching effect on the leaf margins.

The primary leaves of plants treated with cell suspensions of both isolates started turning chlorotic after five days and had fallen off by the twentieth day after application of the cell suspensions. This was one week earlier than falling off of the primary leaves on the other treatments including the control plants. Autoclaving reduced both the effect of bacteria cell suspensions on development of rust pustules and also their phytotoxicity on the treated plants.

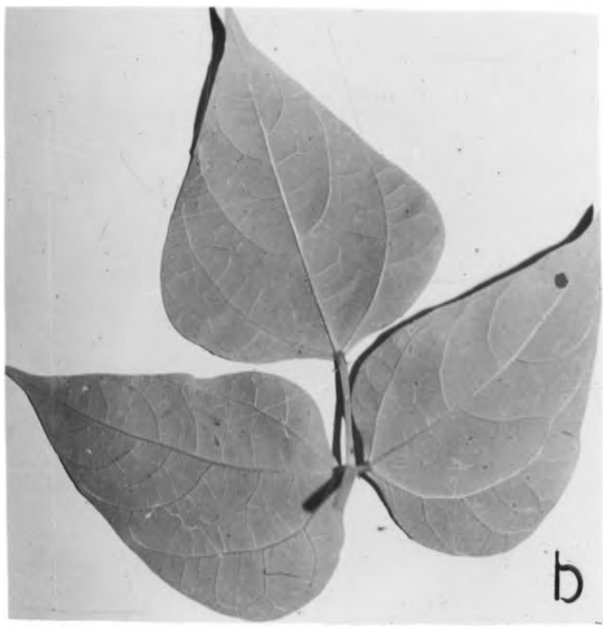


Plate 2
Effect of unautoclaved cell suspensions of *Bacillus* sp isolate CA5 on the morphology of a French bean leaf 17 days after application.
(a) Malformed leaf.
(b) Leaf from untreated plants.

4.2 SEED TREATMENTS

4.2.1 Effect of fungicide treatments on seed germination in the laboratory.

There were significant differences in germination of seeds treated with different fungicides when tested 24 hr after treatment (Table 5). Seeds treated with Raxil 040 had the highest germination mean value of 85.33 %. This was significantly different (P =0.05) from the untreated control seeds with 81.11 %. Real and Anvil treated seeds were not significantly different with mean germination values of 84.33 and 83 % respectively. Baycor and Saprol treated seed were significantly different (P = 0.05) with 81.22 and 80 % mean germination respectively. Raxil 025 had the least germination mean value of 76.66 %.

After storing for two weeks seed treated with Baycor had the highest germination mean value of 78.77 % which was not significantly different from a mean of 78.22 % germination of seeds treated with Anvil. Seeds treated with Raxil 040 had the lowest germination mean value of 55.77 % followed by Raxil 025 with a mean value of 59.22 % germination. The control seeds had a mean germination value of 77.77 %. A comparison of the mean values at 24 hr and after storing the treated seed for two weeks showed that significant differences existed. Seeds treated with Raxil 040 which had the highest value of 85.33 % after 24 hr had the lowest value of 55.77 % after storing for two weeks. This was a decline of 29.56 %. Seeds treated with Raxil 025 had the lowest value of 76.66 and 59.22 % at 24 hr and after storing for two weeks respectively. Seeds treated with Real had 84.33 % germination at 24 hr and had 65.22 % after two weeks.

Table 5 : Analysis of the effect of different fungicides on seed germination in the laboratory 24 hours and two weeks after treatment

Treatment	Mean % germination (24 hr)		Mean % germination (2 wk)	
Raxil 040	85.33	a	55.77	e
Real	84.33	a	65.22	c
Anvil	83.00	abc	78.22	a
Baycor	81.22	bc	78.77	a
Water	81.11	bc	77.77	ab
Saprol	80.00	cd	76.11	b
Raxil 025	76.66	d	59.22	d
Mean	81.66		70.15	
S. E	1.22		0.68	
CV	4.47		2.92	

Any two means followed different letters of the alphabet along the columns are significantly different according to Duncan's Multiple Range Test (P = 0.05).

When evaluated 24 hr after treatment there did not seem to be any direct relationship between the active ingredient of the fungicide used and the mean germination values obtained. For example both Raxil 040 and Raxil 025 are Tebuconazoles but at 24 hr the former had the highest mean value of 85.33 % and the later had the lowest and significantly different mean value of 76.66 %.

After storing seed for two weeks there seemed to be a relationship between the formulation and the active ingredient of the chemical used and the mean germination values obtained. Raxil 040, Raxil 025 and Real which are formulated as flowable concentrates had the three lowest germination values of 55.77, 59.22 and 65.22 % respectively at two weeks.

Anvil, Baycor and Saprol, formulated as emulsifiable concentrates had consistently high mean germination values with only slight variations between the figures obtained at 24 hr and two weeks.

4.2.2 Effect of fungicide dressing on seed germination in the greenhouse

Seeds treated with Anvil, Baycor and Saprol germinated normally and were not different from the control. Seeds treated with Raxil 025, Raxil 040 and Real had suppressed germination. Their cotyledons emerged later than in the control plants and leaf opening was delayed even after emergence. The plants initiall. had short and sturdy thick stems. Stem elongation was severely affected and the plants remained short for extended periods. Their leaves formed slowly were dark green

in colour and were wrinkled with a rough surface. However, the suppression lasted until the expansion of primary leaves after which normal growth resumed and progressed well (Plate 3).

Plate 3
Effect of seed dressing with fungicides on germination and plant development.
(a),(b) and (c) shows depressed germination and development of seeds treated with Rakil 025, Rakil 040 and Real respectively.
(d) Normal development of the control plants.

4.2.3 Effect of seed dressing with fungicides on delaying infection of beans by rust in the greenhouse.

Plants were assessed for infection eight days after inoculation. Assessment was done using the standard CIAT scale (Fig 1). All plants were infected except those treated with Anvil at the higher rate of 3ml /L. However the plants succumbed after a second inoculation was carried out seven days after the first one.

On the first day of assessment which was eight days after the initial inoculation most plants had only little infection per leaf. Plants from seed treated with Real had the highest infection with a mean of about 1 % severity. Plants from seed treated with Raxil 025 had the lowest initial infection with a mean of 0.57 % (Table 6).

The second assessment was done on the 14 th day after the initial inoculation and seven days after the second inoculation. Plants from seed treated with Saprol had the highest infection of 2.93 % which was significantly different from all other treatments (P =0.05). Plants from seed treated with Baycor, Raxil 025, Raxil 040 and the control had similar infection rates of 2 %. The lowest infection of 1.66 % was recorded from the plants raised from Anvil treated seed. The overall average score of 2.22 % obtained on this second assessment was significantly different from the 0.98 % obtained on the first assessment.

The third assessment for infection was done 21 days after the initial inoculation. Plants from seed treated with Baycor had the highest infection score of 8.23 % but this was not significantly different (P =0.05) from plants of seed treated

with Saprol which had an infection of 7.47 %. All other treatments had infection rates ranging between 4.83 to 5.83 %. The overall average infection score of 6.05 % on this assessment was significantly different from the 2.22 % recorded on the second assessment.

The fourth assessment was done on the 28th day after the initial inoculation. Plants from seeds treated with Baycor had the highest infection with 10.66 % which was significantly different (P =0.05) from all other treatments. Plants from seed treated with Saprol, Raxil 025 and Raxil 040 had infections ranging between 6.7 to 8 %. Plants from seed treated with Anvil had a mean score of 5.27 % which was significantly different from the control plants with 5 % and from plants of seed treated with Real which had a mean of 5.8 % severity. The overall mean score of 6.84 % on this assessment was not significantly different from 6.05 % recorded on the third assessment.

The fifth and last assessment for infection was done on the 35th day after the initial inoculation. Plants from seed treated with Baycor and Saprol had the highest infection of 11.8 and 10.7 % severity respectively. Plants from seed treated with Raxil 025, Raxil 040 and Real had a mean severity between 7.5 and 8.2 % and none was significantly different from the control plants which had 7.5 %. Plants from seed treated with Anvil had the lowest severity score of 5.44 %. The overall mean score of 8.4 % was significantly higher than the 6.84 % recorded on the fourth assessment.

Disease progress curves were plotted for the different fungicides (Fig 3,4,5). From the curves a rapid increase of

disease is observed between the 7th and 21st day after the initial inoculation. The small gradient between the 21st and 35th day corresponds to the slow disease increase due to the senescence of the more severely infected primary leaves.

Table 6: Disease severity data (%) on French beans from fungicide treated seeds twice inoculated with rust in the greenhouse at Kabete.

	Days after initial inoculation				
Treatment	8	14	21	28	35
Anvil	0.87 c	1.66 c	5.16 b	5.27 c	5.44 c
Baycor	1.00 bc	2.03 bc	8.23 a	10.66 a	11.83 a
Saprol	0.83 bc	2.93 a	7.47 a	8.00 b	10.66 a
Rakil 025	0.57 c	2.03 bc	5.83 b	6.53 bc	8.17 b
Rakil 040	1.43 a	2.16 abc	5.50 b	6.66 bc	7.11 bc
Real	1.17 ab	2.70 ab	5.36 b	5.83 c	8.11 b
Untreated	1.00 bc	2.00 bc	4.83 b	5.00 c	7.50 b
Mean	0.98	0.21	6.05	6.84	8.40
S.E	0.14	0.26	0.48	0.57	0.62

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncans Multiple Range Test (P = 0.05).

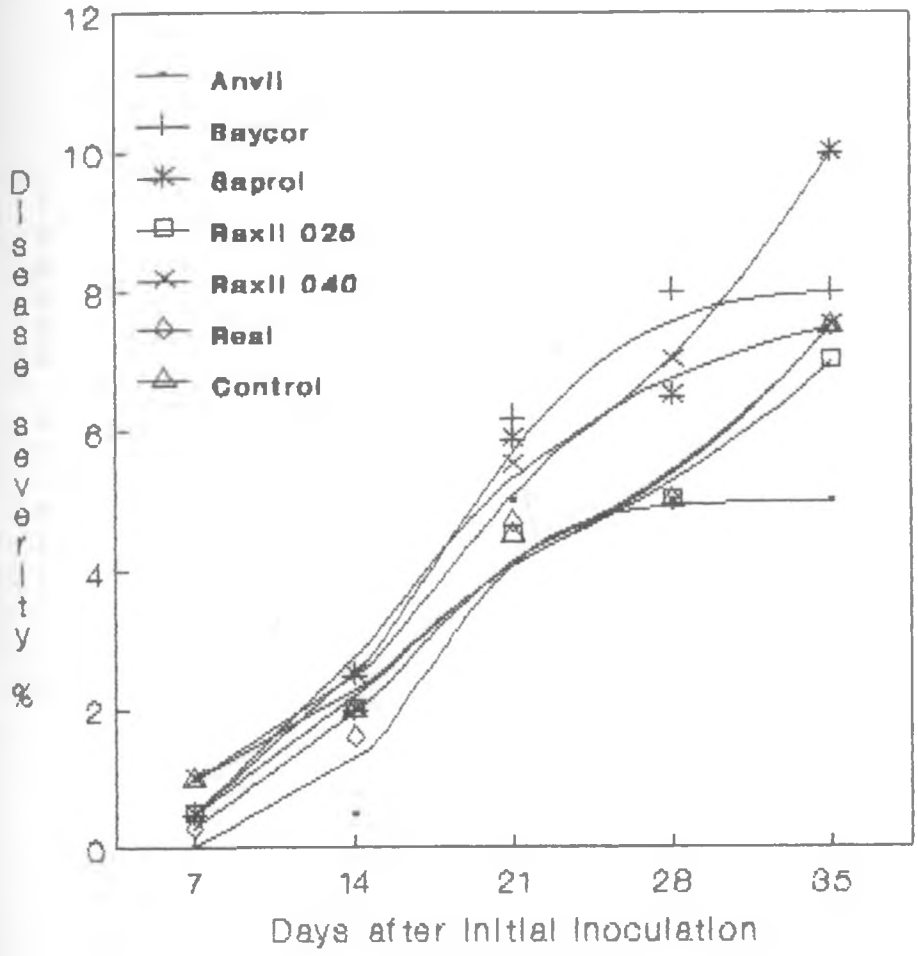


Fig 3:Disease progress curves:plants from seed treated with different fungi--cides at the high rate at Kabete

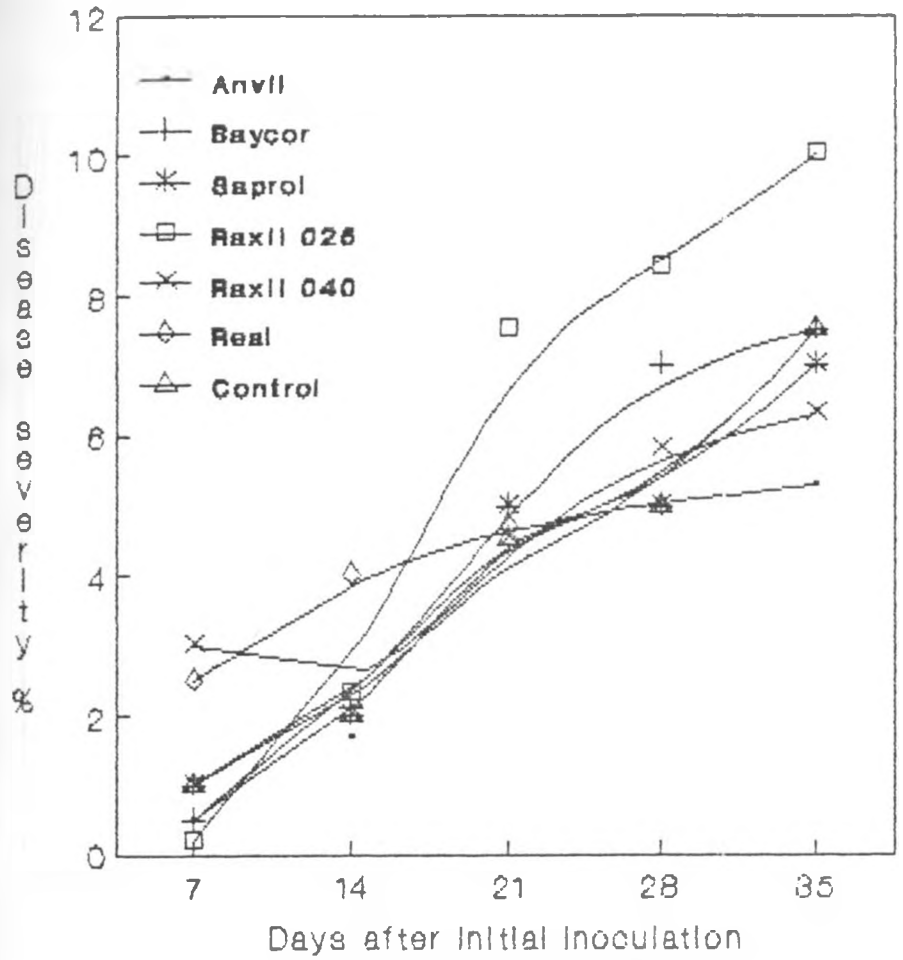


Fig 4: Disease progress curves: plants from seed treated with six different fungicides,medium rate at Kabete.

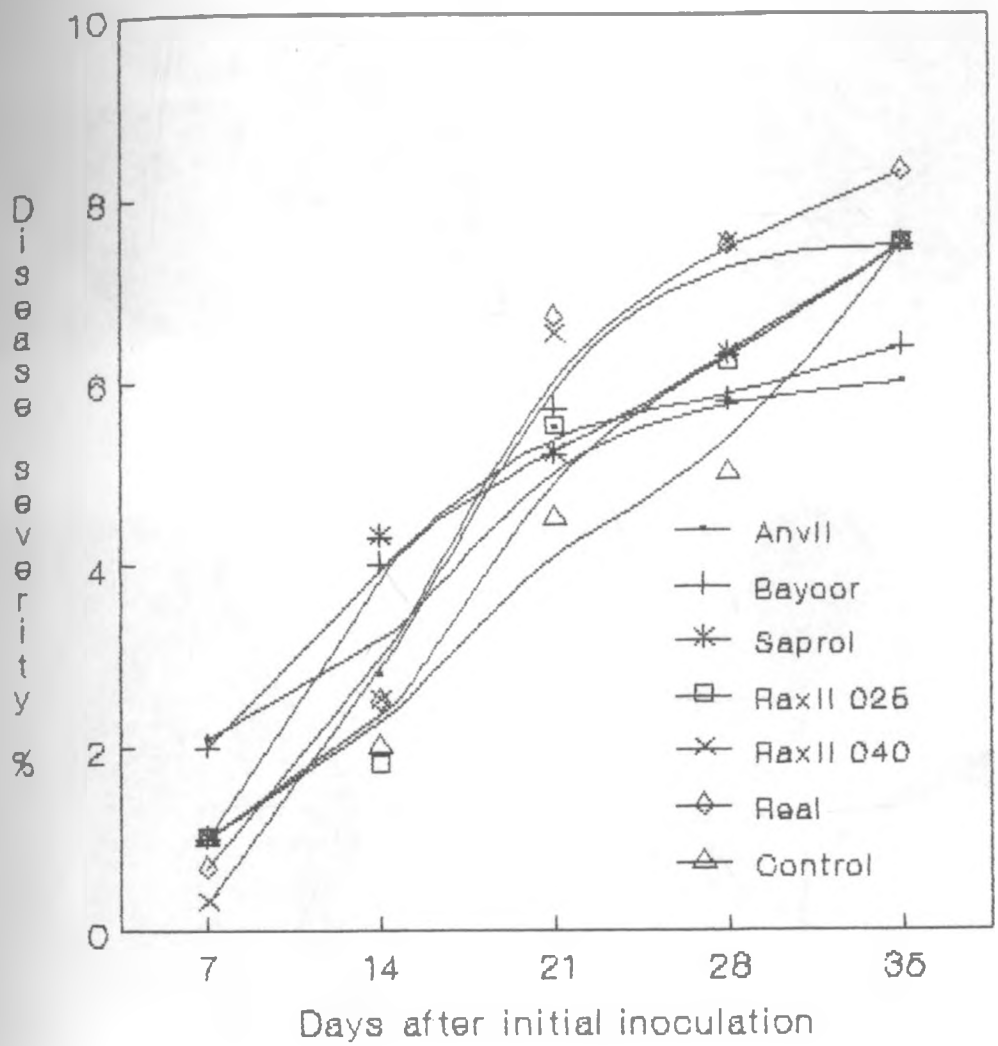


Fig 5:Disease progress curves: plants from seed treated with six different fungicides,low rate at Kabete greenhouse

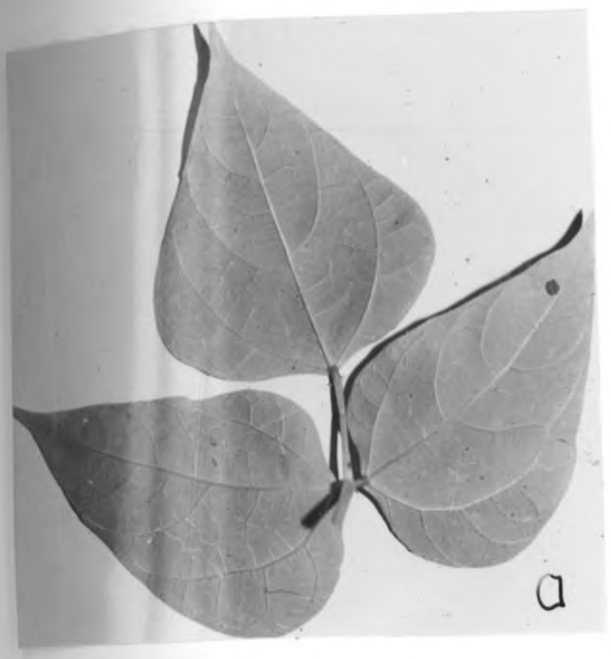


Plate 4
Estimation of bean rust severity using the Modified Cobb scale.
(a) Uninfected leaf.
(b) 5 % severity
(c) 1[illegible] % severity
(d) 4[illegible] % severity

4.2.4 Effect of fungicide treatment on seed germination in the field

Germination count was carried out fourteen days after planting at both Kabete and Naivasha. In each plot all the plants which were emerged with their cotyledons open were counted. Germination was over 80 % in all treatments but plant emergence and development was suppressed in seeds treated with Raxil 025, Raxil 040 and Real. In these treatments only a few seedlings had their cotyledons fully opened while most were found to be at the crook head stage. The plants which had emerged had thick sturdy stems and dark green wrinkled leaves. This observation was consistent with what was seen in the greenhouse where the same fungicides were evaluated.

4.2.5 Effect of seed dressing with fungicides on rust infection of beans in the field.

Naivasha site

The first assessment for infection was done seven days after the initial inoculation. All bean plants had a rust severity score of more than 5 % except the plants from seed treated with Real and the control which had 4 and 3 % respectively (Table 7).

On the second assessment which was seven days later, infection severity had only increased slightly. Plants from seed treated with Saprol had the highest severity score of 10.54 %. The protected control plants had the lowest score of 5 %.

The third assessment for infection was done 21 days after the initial inoculation. The severity score had increased

drastically and was over 90 % in all treatments except on the protected control plots where the increase was from 5 to 25 %. On the control plots this increase was inspite of the fact that Mancozeb was being applied weekly to protect the plants from infection. By the third week the most severe infection was on the primary leaves although the first two trifoliate leaves had developec and were bearing infection of about 10 %.

All plants except those in protected control plots had the primary leaves falling off between the third and the fourth week after attaining 100 % severity. On the fourth week therefore assessment scores were taken from the first trifoliate leaves which were bearing an average of 80 % severity. By the fifth week infection on the first trifoliate leaves had risen to 99 % and they had started falling. It was observed that the first and second trifoliate leaves differed only slightly in age and disease development on them was similar. Therefore the first and the second trifoliate leaves fell simultaneously or the latter defoliated slightly after the first one.

On the sixth week severity data were taken from the third trifoliate leaves which had an average of 50 - 55 % on all treatments. Harvesting started on the seventh week after the initial inoculation when infection on the third trifoliate leaves had risen to 80 %. This increased to 95 % by the 9 th week and the third trifoliate leaves fell off.

Table 7: Disease severity (%) on french beans from seeds treated with different fungicides at Naivasha.

	Days after initial inoculation							
Treatment	7	14	21	28	35	42	49	56
Anvil	6.5	7.1	99.0	80.0[b]	99.0	52.5[b]	80.0	95.0
Baycor	7.4	8.0	97.6	80.0	99.0	52.5	80.0	95.0
Saprol	7.5	10.5	93.3	80.0	99.0	52.5	80.0	95.0
Raxil 025	5.8	9.9	99.0	80.0	99.0	52.5	80.0	95.0
Raxil 040	6.3	7.2	97.6	80.0	99.0	52.5	80.0	95.0
Real	4.0	6.6	99.0	80.0	99.0	52.5	80.0	95.0
Untreated	6.4	8.9	93.3	80.0	99.0	52.5	80.0	95.0
Mancozeb	3.0	5.0	25.0	40.0	55.0	65.0	80.0	95.0

[b] Scores along these columns are lower than on the previous columns because they were recorded from a new leaf after the previous most infected one fell.

Kabete site

Results obtained for Kabete showed that the disease was not severe. On the first assessment which was seven days after the initial inoculation only very little infection was observed (Table 8). During the second week the highest infection score was 1.7 % recorded from plants treated with Raxil 025 and Raxil 040. The control plants which were protected with Mancozeb did not show any infection.

On the third assessment which was 21 days after the initial inoculation the highest infection was 3 % observed on plants from seed treated with Raxil 025. By the sixth week after inoculation the maximum severity recorded was 10 % from plants of seed treated with Raxil 040 and Real. Plants from seed treated with Anvil, Baycor, Saprol and Raxil 025 had infection in the range of 8.9 - 9.5 %.

By the end of the eighth week plants from seed treated with Baycor, Raxil 025, Raxil 040 and Real had infection averaging 15 % while those from seed treated with Anvil and Saprol averaged 12.5 %. The protected control plants did not show any infection throughout the season.

Table 8: Disease severity (%) on French beans from seeds treated with different fungicides at Kabete.

Treatment	Days after initial inoculation							
	7	14	21	28	35	42	49	56
Anvil	0.3	1.0	2.0	7.5	8.0	8.9	10.0	12.5
Baycor	0.3	0.8	2.5	8.2	8.5	9.2	12.5	15.0
Saprol	0.3	1.1	2.5	7.0	7.5	9.0	10.0	12.5
Raxil 025	0.0	1.7	3.0	7.5	8.2	9.5	12.5	15.0
Raxil 040	0.2	1.7	2.7	7.0	7.6	10.0	12.5	15.0
Real	0.3	1.0	3.0	6.8	7.5	10.0	12.5	15.0
Untreated	0.3	0.3	4.0	6.2	6.9	8.2	10.0	12.5
Mancozeb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

4.2.6 Disease progress curves

Two observations were made from the data obtained. One is that at Naivasha disease was severe and reached an optimum level of 100 % while at Kabete it was mild and maximum severity observed was only 10 %. To enable plotting of comparable disease progress curves for both sites data was classified on basis of which leaf was scored (Table 9a, 9b).

At Naivasha disease progressed in a similar pattern in all the treatments except in the control plots which were protected from infection by Mancozeb sprays. Data recorded from the unprotected control plots was used to plot the disease progress curves as a representative of the Naivasha site (Fig 6a, b, c). At Kabete data recorded showed the pattern of disease progress to be similar in all treatments except in the protected control plants which did not get infected. Data taken on three different leaves of the unprotected control plots (Table 9b) were used to plot the disease progress curves as a representative of the Kabete site (Figure 6a,b,c).

The curves were plotted so as to enable comparison of disease progress on the primary leaves at both sites (Fig 6a), first trifoliate leaves (Fig 6b) and the third trifoliate leaves (Fig 6c). It can be observed from the comparative curves that while all leaves reached a maximum infection of 100 % severity at Naivasha, the maximum for Kabete was 10 %.

Fig 6a shows that between the seventh and fourteenth day there was only very slight increase in infection at both sites. At Naivasha the infection increased only from 6.4 to 8.86 % while at Kabete it increased from 0.3 to 0.8 %. However the increase

between fourteenth and twenty first day was very significant for Naivasha, where it reached over 90 % while at Kabete it increased to 4 %.

The sharp increase at Naivasha could have been due to the favourable weather conditions and also due to the substantial infection which had been initiated earlier unlike at Kabete where the weather was unfavourable and only little infection had been initiated.

Fig 6b shows that there was a sharp increase in infection from 10 to 80 % on the first trifoliate leaves between the twenty first and the twenty eighth day after inoculation. At the same time the curves show only a slight change in gradient at Kabete where the increase was from 4 to 6.2 %. Due to severe infection at Naivasha the leaves fell off after 42 days while at Kabete the infection was increasing slightly per week and reached 12.5 % after 56 days.

Fig 6c shows that on the third trifoliate leaves infection started a week earlier at Naivasha than at Kabete where the initial score was 2.5 %. The subsequent increase in infection was steady at Naivasha where it reached 95 % after 56 days while at Kabete the severity had reached only 15 % after 56 days.

Table 9a: Disease severity (%) recorded from three different leaves of French beans in the unprotected control plots at Naivasha.

	Days after initial inoculation						
Leaf taken	7	14	21	28	35	42	49
Primary	6.4	8.9	93.3	10.0			
$1^{st}+2^{nd}$ trifoliate	ND	5.5	10.0	80.0	99.0	100	
3^{rd}++ trifoliate	ND	ND	5.0	7.5	25.0	52.5	80.0

ND means the leaf being scored had not developed at this date and so no data were recorded.

Table 9b: Disease severity (%) recorded from three different leaves of French bean plants in the unprotected control plots at Kabete.

	Days after initial inoculation							
Leaf taken	7	14	21	28	35	42	49	56
Primary	0.3	0.8	4.0	10.0				
$1^{st}+2^{nd}$ trifoliate	ND	2.0	4.0	6.2	6.9	8.2	10.0	12.5
3^{rd}+++ trifoliate	ND	ND	0.0	2.5	4.0	7.5	12.5	15.0

ND means the leaf being scored had not developed at this date and no data were recorded.

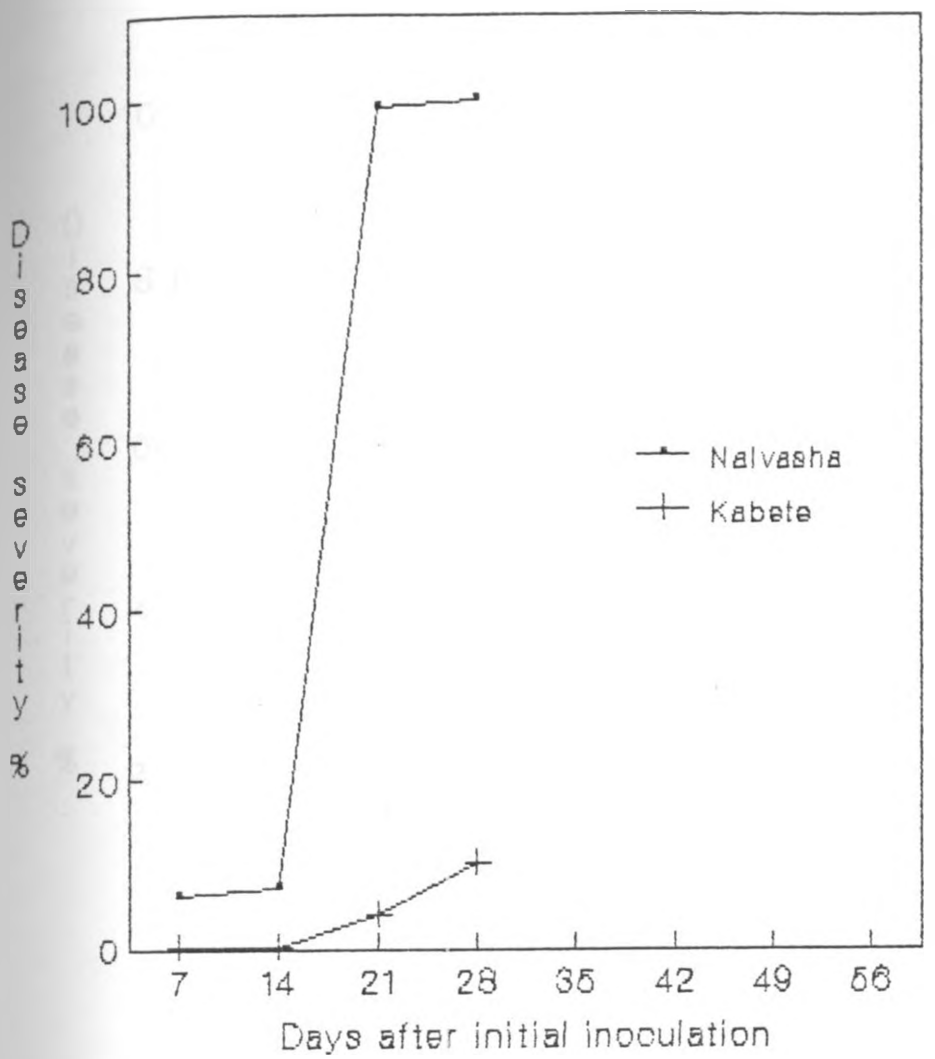


Fig 6a:Disease progress curves showing comparison of data from primary leaves of control plots at Kabete and Naivasha

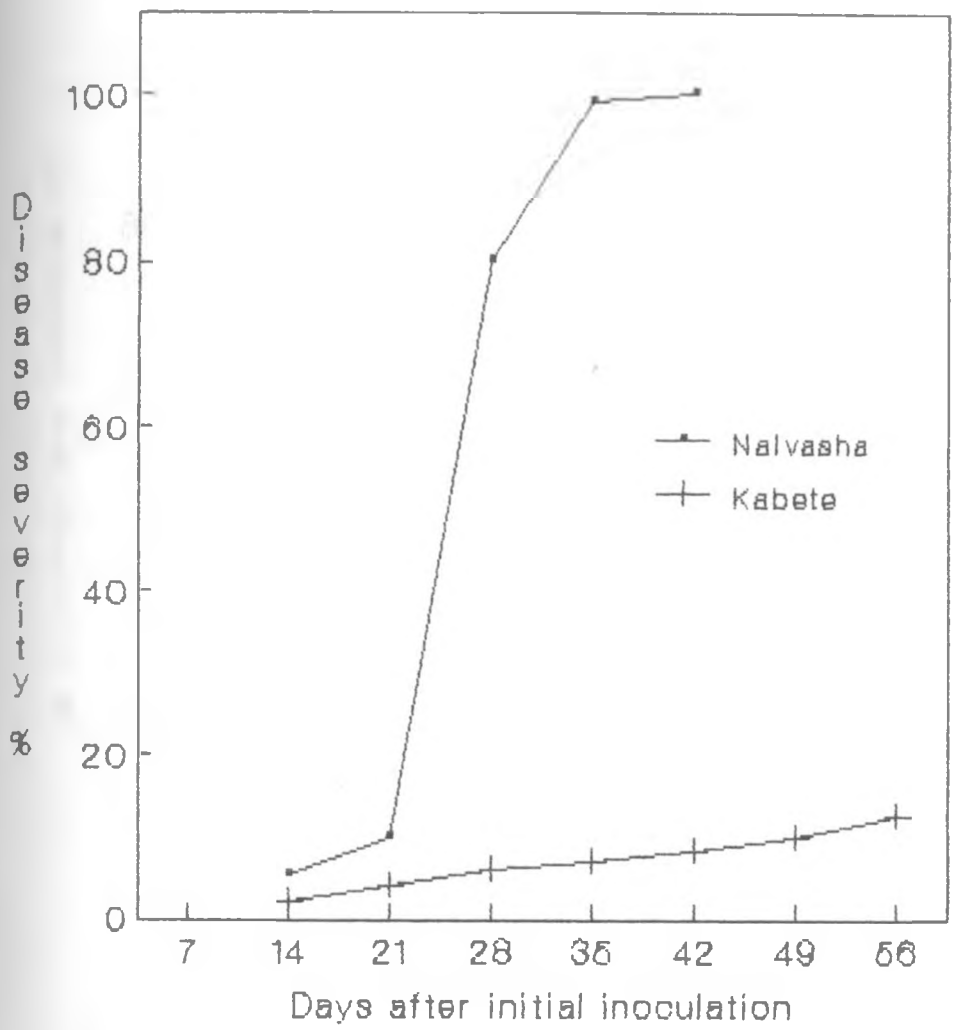


Fig 6b:Disease progress curves showing comparison of data from 1 st trifoliates of control plots at Kabete and Naivasha

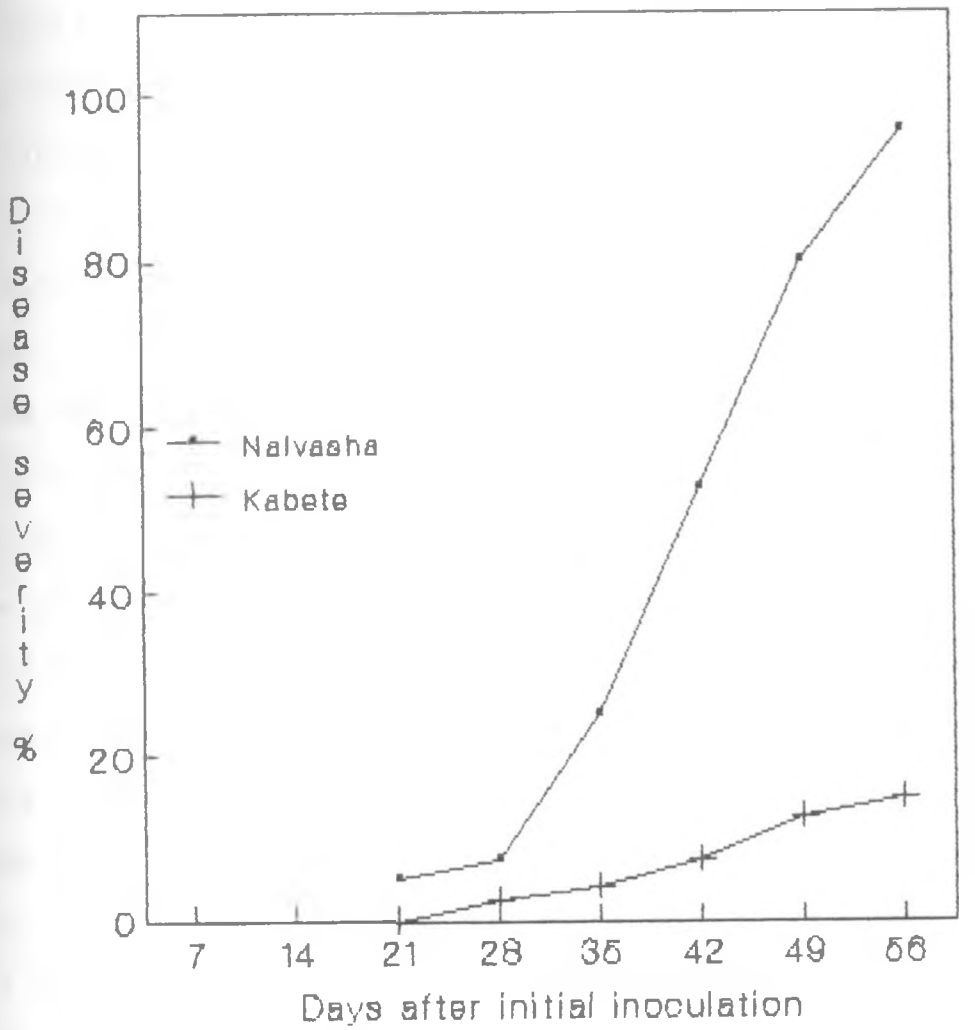


Fig 6c: Disease progress curves showing comparison of data from 3 rd trifoliates of control plots at Kabete and Naivasha.

4.2.7 Area Under the Disease Progress Curves (AUDPC)

At each site disease progress was observed on the primary, first and third trifoliate leaves (Fig 6a,b,c). Analysis of AUDPC showed that at Naivasha more area was covered by rust on the third trifoliate leaves (15 units) than on the first trifoliate leaves (13 units). The primary leaves had 10.8 units which was not significantly different from the first and third trifoliate leaves.

At Kabete the primary leaves had significantly less disease (0.69) than the first and the third trifoliate leaves which had 2.97 and 2.38 units respectively. The first and third trifoliate leaves did not differ significantly at P = 0.05.

A high AUDPC was realised for the primary leaves at Naivasha because infection had set in earlier than on the other leaves. Falling off of the primary leaves was also delayed and this enabled collection of data for an extended period. At Kabete the primary leaves fell between the third and fourth week due to normal senescence and only little infection had been realised by then (Table 9c).

Table 9c: Analysis of Area Under Disease Progress Curves for data recorded from three different leaves at Naivasha and Kabete.

Leaf scored	Naivasha	Kabete
Primary	10.88 a	0.70 a
1st trifoliate	13.11 a	2.98 b
3rd trifoliate	15.10 a	2.38 b
Mean	13.00	2.02

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncans Multiple Range Test (P=0.05)

A comparison of AUDPC figures for the two sites (Table 9d) showed that more area of foliage was covered by infection at Naivasha than at Kabete.

Table 9d: Analysis of AUDPC on site basis

Site	Mean AUDPC
Kabete	2.02
Naivasha	13.01
Mean	7.50

4.2.8 DISEASE PROGRESS RATES

The rate of disease progress was calculated using the Gompertz model (Berger,1981).

Formula used was:

$$K = \frac{\text{Gompit } y_2 - \text{Gompit } y_1}{T_2 - T_1}$$

Where K is the rate of disease progress

y is the severity score observed at time T1, T2,etc

Gompit y = - ln(-ln y)

T is the time at which data is collected.

The rates of disease at Naivasha were low at the initial stages of disease regardless of the leaf under consideration and increased as the area covered by disease increased. The initial rates of progress on the primary, first and third trifoliate leaves were 0.005, 0.033 and 0.021 units respectively. After two weeks the rate of progress on the first trifoliate leaves had increased to 0.44 while after four weeks the rate of progress on the third trifoliate leaves had increased to 0.24 (Table 9e).

At Kabete the rates of disease progress remained consistently low throughout the experiment period. On the primary leaves the initial rate of disease progress was 0.084. The initial rate on the first trifoliate leaf was 0.028 which later decreased to 0.0095 by the fourth week. On the third trifoliate leaf the initial rate was 0.019 while after three weeks it was 0.013 (Table 9f).

Table 9e: Week to week rates of disease progress on the primary, first and third trifoliate leaves of French beans in the untreated seed plots at Naivasha.

	Days after initial inoculation in weeks						
Leaf taken	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Primary	.01	.08					
1st+2nd trifoliate		.03	.33	.44			
3rd ++trifoliate			.02	.09	.11	.15	.24

NB: Days After Initial Inoculation have been transformed into weeks, e.g between 7th and 14 th day is Week 1 - 2.

Table 9f:Week to week rates of disease progress on the primary, first and third trifoliate leaves of French beans in the untreated seed plots at Kabete.

	Days after initial inoculation in weeks						
Leaf taken	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Primary	.084	.048					
1st+2nd trifoliate		.028	.021	.006	.010	.012	.014
3rd ++ trifoliate			.019	.031	.031	.013	

NB: Days after initial inoculation have been transformed into weeks, e.g. between 7th and 14th day is week 1-2.

4.3 INCIDENCE

Two kinds of incidence were considered. One was the proportion of plants bearing infection out of the total number of plants assessed. The other was the number of leaves bearing infection per assessed plant.

Naivasha site

At the Naivasha site where disease was prevalent 100 % of the plants assessed were infected. At the first scoring date only the two primary out of the four leaves present were infected (Table 10). The first and second trifoliate leaves were not fully developed and did not show any infection. On the second assessment each plant had five leaves (the third trifoliate leaves had opened) out of which three were infected. It was observed that after the third trifoliate, the next three trifoliate leaves developed at about the same time and matured simultaneously. Therefore during the third date of assessment done 21 days after inoculation each plant had eight leaves six of which were infected.

Defoliation either due to infection or due to normal senescence occurred between the third and fourth week about 23 days after the initial inoculation. On all except the protected control plots the first to fall off were the primary leaves which were bearing maximum infection. When incidence data were being recorded on the fourth week, the average additive count was 17 leaves per plant. Two primary leaves had fallen off and the next three trifoliate leaves were about to fall. This left only about 12 leaves which were of any photosynthetic value to the plant.

Ten of those twelve leaves were observed to be infected.

By the fifth week, the trifoliate which previously had an infection of 80 % severity had fallen off. This left the plant with two leaves less from below. At the same time, the plant had developed two new leaves at the top, thus restoring the original number of 12 leaves which were contributing photosynthetically and raising the additive leaf count to 19 per plant. The newly formed leaves had not developed any infection.

By the sixth week young leaves were developing where others had fallen off earliest. All the twelve mature leaves present were infected with the oldest of them bearing a maximum severity of 52.5 % (Table 7).

By the seventh week after initial inoculation three new leaves formed where defoliation had occurred earliest had matured raising the additive leaf count to 22 per plant. On the protected control plants falling off of the primary leaves was delayed and occurred after 28 days. The first and second trifoliate leaves fell between the sixth and seventh week after attaining about 80 % infection severity.

Kabete site

At Kabete infection was minimal and progress poor. Other than the primary leaves which fell due to normal senescence no other leaf fell during experimentation due to rust infection.

Table 10: Proportion of infected bean leaves in plots planted with French bean seed treated with different fungicides at Naivasha

	Days after initial inoculation						
	7	14	21	28[a]	35[b]	42[b]	49[c]
Treatment	Wk 1	Wk 2	Wk3	Wk 4	Wk 5	Wk 6	Wk 7
Anvil	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Baycor	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Saprol	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Rakil 025	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Rakil 040	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Real	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Untreated	0.50	0.60	0.75	0.88	0.89	1.00	0.86
Mancozeb	0.50	0.60	0.75	0.59	0.73	0.84	0.95

[a] By this date five leaves had dropped off except on the plots sprayed with Mancozeb where no leaf had fallen off.

[b] By this date seven leaves had dropped off except on plots sprayed with Mancozeb where only two had fallen off.

[c] Three new leaves had formed where others had fallen off except on plots sprayed with Mancozeb where by now only four leaves had fallen.

4.4 YIELD ANALYSIS

Naivasha site

The highest yield of 11.1 kg per plot which is equivalent to 9250 kg/ha was obtained from the unprotected control plots (Table 11a). Anvil, Saprol and Raxil 025 treatments had yield between 7.5 - 9.2 kg/plot which is equivalent to 6250 - 7667 kg/ha and were not significantly different (P =0.05) from the protected control plots which had 9.9 kg/plot. Baycor, Real and Raxil 040 had yield ranging 6.3 - 6.96 kg/plot which were significantly lower than all other treatments (P =0.05). When analysed at P = 0.01 no treatment was significantly different from the control.

Kabete site

There were significant differences between yields obtained from plots with different treatments (Table 11a). The protected control plots had the highest yield of 19.2 kg /plot equivalent to 16000 kg/ha which was significantly different from all other treatments (P =0.05). Raxil 040, Baycor and Saprol had yields ranging 17.3 - 17.6 kg/plot which is equivalent to 14416 - 14666 kg/ha and were not significantly different (P = 0.05) from the unprotected control plots which had a mean yield of 18.3 kg/plot. Raxil 025 had a significantly low yield of 15.6 kg/plot (P = 0.05).

Table 11 a:Analysis of yield (kg/plot) of French beans raised from seed treated with different fungicides before planting at Kabete and Naivasha.

Treatment	Naivasha	Kabete
Untreated seeds	11.13 a	18.33 ab
Mancozeb sprays	9.90 ab	19.20 a
Anvil	9.23 ab	16.23 cd
Saprol	8.33 ab	17.33 abcd
Raxil 025	7.50 ab	15.57 d
Baycor	6.96 b	17.40 abcd
Real	6.80 b	16.56 bcd
Raxil 040	6.33 b	17.60 abc
Mean	8.28	17.28
S.E	1.13	0.57

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncan's Multiple Range Test (P =0.05).

It was found that differences between sites were highly significant at both 5 and 1 % levels (Table 11b). Mean yield at Kabete was twice as much as at Naivasha.

Table 11b: Analysis of mean yield (kg/plot) on site basis.

Site	Mean yield (kg/ha)
Kabete	17.28
Naivasha	8.28
LSD 0.05	0.91
LSD 0.01	1.23

4.5 EVALUATING YIELD DEPENDING ON THE STAGE OF INFECTION

4.5.1 Disease progress at Naivasha

Data for the plots inoculated at the primary leaf stage were initially recorded from the primary leaves until they fell after about five weeks (Table 12). Inoculum on all other sets of treatment plants was placed directly on trifoliate leaves. Therefore data from such plants was mainly recorded from the trifoliate leaves which also contribute more to the nutritional demands of the plant and hence their infection was of more significance. On all treatments falling off of trifoliate leaves was observed to be a function of both earliness and severity of infection.

On the plants inoculated at the growth stages V3 (1 st trifoliate) and V 4 (3 rd trifoliate), the first trifoliate leaf fell between the eighth and ninth week after planting. The leaves had attained over 90 % severity after bearing infection for between five and six weeks. The first trifoliate leaves of plants inoculated at the primary leaf stage V 2 did not get infected and thus were still healthy and in place when those of plants inoculated at stage V3 (1 st trifoliate) and V 4 (3 rd trifoliate) fell off eight weeks after planting.

On plants inoculated at stage R5 (pre-flowering) and R6 (flowering) the first trifoliate leaves fell nine weeks after planting. The leaves had attained over 80 % severity after bearing infection for between five and six weeks.

On plants inoculated at other growth stages except V 2 the primary leaves fell during the fourth week (23 - 28 days) after opening. This was presumed to be due to normal senescence as

infection on them was only slight. On plants inoculated at the growth stage V 2 the primary leaves attained 80 % severity and started falling off 35 days after inoculation.

On the plants inoculated at the pod formation stage (R 7) no trifoliate leaves had fallen by the tenth week after planting. This was also the case on plants in the control plots which were protected from infection throughout the growing period (Table 12).

Table 12: Disease severity (%) on French bean leaves inoculated at different stages of growth at Naivasha.

Stage of inoculation	Days after planting							
	21	28	35	42	49	56	63	70
V2	5.0	22.7	33.5	50.0	80.0	5.0[c]	10.0	20.0
V3	5.0	27.9	49.2	52.0	68.5	93.0	46.0[c]	63.0
V4		4.0	30.5	43.7	69.7	93.4	42.0[c]	79.2
R5			1.7	29.6	49.5	63.7	88.0	47.0[c]
R6			5.0	16.5	19.8	53.2	82.0	35.0[c]
R7			5.0	7.5	12.0	16.4	25.0	52.5
Mancozeb spray				5.0	5.0	5.0	10.0	12.5

V is for stages of growth in the vegetative phase while R is for stages in the reproductive phase.

V2 is primary leaf stage.

V3 is first trifoliate leaf stage.

V4 is third trifoliate leaf stage.

R5 is pre-flowering stage.

R6 is flowering stage.

R7 is pod formation stage.

[c] Indicate that this score was observed on a different leaf after the previous one fell due to infection except stage V2 where previous scores were being taken on primary leaves.

The spread of disease was observed to be most slow when plants were inoculated at the primary leaf stage. On the first incidence assessment day only two of the three leaves present on stage V 2 plants were infected (Table 13).

On the second assessment three of the five leaves present were infected with the primary leaves bearing the highest mean severity of 22.74 %. By the eighth week after planting 100 % of foliage on plants inoculated at stages V 3 and V 4 showed infection. Plants inoculated at stage V 4 showed the spread of disease with more acceleration than plants inoculated at stage V 2. This was due to the fact that infection had been established on more foliage (more surface area) and more severely on plants inoculated at stage V4 than on plants inoculated at stage V2. The spread of disease on the plants inoculated at stage R 5 (pre-flowering) was fast as infection established on leaves at the upper mid-section of the canopy spread quickly to the leaves placed laterally and below them.

Table 13: Proportion of infected bean leaves in French bean plots inoculated at different stages of growth at Naivasha.

Stage of inoculation	Days after planting						
	21	28	35	42	49	56	63
V2	0.67	0.60	0.56	0.77	0.84	0.89[b]	0.77
V3		0.40	0.55	0.77[a]	0.84	0.89	0.86
V4			0.44	0.77[a]	0.84	0.89	0.86
R5				0.18[a]	0.37	0.53	0.68
R6					0.21	0.37	0.45
R7						0.21	0.32
Mancozeb						0.21	0.32

[a] At this point two primary leaves fell off due to normal senescence

[b] At this point the primary leaves fell off due to infection

V is stages of growth in the vegetative phase while R is for stages of growth in the reproductive phase.

V2 is primary leaf stage.

V3 is first trifoliate leaf stage.

V4 is third trifoliate leaf stage.

R5 is pre-flowering stage.

R6 is flowering stage.

R7 is pod formtion stage.

4.5.2 Disease progress at Kabete

Disease establishment was low and progress poor. On the eighth day plants inoculated at the primary leaf stage (V 2) had a maximum infection of 5 pustules per leaf (Table 14). This infection increased to 2 % after one week and reached 18 % by the fourth week. Plants inoculated at stage V 2 started defoliating six weeks (42 days) after opening having attained a maximum infection of 57 % severity. The primary leaves of plants inoculated at other growth stages started falling off 28 days after opening due to normal senescence. On all treatments no trifoliate leaf fell throughout the growing season (Table 15).

Incidence assessment at Kabete showed the pattern of disease spread to be the same as at Naivasha. However due to differences in the severity of disease it took longer for any appreciable area and amount of foliage to be infected at Kabete than at Naivasha.

Table 14: Disease severity (%) on French bean plots inoculated at different stages of growth at Kabete.

Stage of inoculation	Days after Planting							
	21	28	35	42	49	56	63	70
V2	0.2	2.0	10.0	18.3	31.0	57.4	29.5[b]	41.0
V2		1.7	5.0	12.5	25.0	47.0	33.0	43.0
V4			3.8	8.3	25.0	41.0	53.0	55.0
R5				1.7	7.8	20.0	37.5	64.0
R6					1.7	5.0	10.0	21.5
R7						1.7	5.0	10.0
Mancozeb						2.5	5.0	6.2

V is for stages of growth in the vegetative phase. R is for the stages in the reproductive phase.

V2 is primary leaf stage.

V3 is first trifoliate leaf stage.

V4 is third trifoliate leaf stage.

R5 is pre-flowering stage.

R6 is flowering stage.

R7 is pod formation stage

[b] This figure was recorded form the first trifoliate leaf after the primary leaf fell.

Table 15: Proportion of infected leaves on French bean plots inoculated at different stages of growth at Kabete

Stage of inoculation	Days after planting						
	21	28	35	42	49	56	63
V2	0.67	0.60	0.55	0.70	0.74	0.74	0.68
V3		0.60	0.55	0.82	0.74	0.74	0.64
V4			0.44	0.82	0.74	0.74	0.90
R5				0.24	0.37	0.58	0.64
R6					0.21	0.37	0.64
R7						0.21	0.32
Mancozeb	0.0	0.0	0.0	0.0	0.0	0.0	0.0

V is for the stages of growth in the vegetative phase while R is for the stages in the reproductive phase of growth.

V2 is primary leaf stage.

V3 is first trifoliate stage.

V4 is third trifoliate leaf stage.

R5 is pre-flowering stage.

R6 is flowering stage.

R7 is pod formation stage.

4.5.3 Area Under the Disease Progress Curves (AUDPC)

Analysis of data from Naivasha showed that plants which were inoculated at growth stages earlier than the pre-flowering phase had an AUDPC of over 10 units which was significantly different from 2.03 units for the control plots (Table 16). The high figure calculated for plants inoculated at growth stage V2 and V3 is not of much significance because only few trifoliate leaves got this high level of infection. On the plants inoculated at growth stages V4 (third trifoliate) and R5 (pre-flowering) the high figure of 13.45 and 13.10 are of significance because they reflect the high level of infection attained on the third trifoliate leaves which are of great importance photosynthetically.

Although a substantial number of trifoliate leaves were infected when plants were inoculated at growth stage R6 only a low level of infection was attained as reflected by the low AUDPC.

At Kabete plants inoculated at growth stages V4 and R5 had the first and second highest AUDPC values of 10.76 and 6.86 respectively. This was significantly different from all other treatments including the control 0.65 units. Inoculation of plants at growth stages R6 and onwards was late and disease did not develop to any significant levels.

Table 16: Analysis of area under disease progress curves (AUDPC) of French beans inoculated at different stages of growth at Kabete and Naivasha.

Stage of inoculation	Naivasha	Kabete
V3	17.22 a	4.51 d
V4	13.45 b	10.76 a
R5	13.12 b	6.86 b
V2	10.41 c	6.31 c
R6	9.30 d	1.84 e
R7	6.25 e	0.73 f
Mancozeb spray	2.03 f	0.65 f
Mean	10.25	4.52
CV	0.93	1.34
S.E	0.25	0.16

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncans Multiple Range Test (P = 0.05).

V is for the stages of growth in the vegetative phase while R is for stages in the reproductive phase of growth.

V2 is primary leaf stage.

V3 is first trifoliate leaf stage.

V4 is third trifoliate leaf stage.

R5 is pre-flowering stage.

R6 is flowering stage.

R7 is pod formation stage.

4.6 YIELD ANALYSIS

Kabete site

Separation of yield means by Duncans Multiple Range Test showed that plots inoculated at different stages except the flowering stage (R6) differed significantly from the control (Table 17a). Plots inoculated at the third trifoliate leaf stage had the lowest yields 13.43 kg/plot which is equivalent to 11072 kg/ha. This represented a loss of 3301.95 kg/ha, equivalent to 22.9 % loss.

Plots inoculated at the pre-flowering (R5) stage had the second lowest yields of 13.5 kg/ha which is equivalent to 11298.6 kg/ha, a loss of 21.4 %. Plots inoculated at stages V2 (primary leaf) and V3 (first trifoliate) had higher mean yield of 14.6 and 15.4 kg/plot respectively but were significantly less than plants inoculated at the pod formation stage (R 7) which had a mean of 16.7 kg/plot (equivalent to 13916 kg/ha) and control plots which had a mean of 16.6 kg/plot.

Naivasha site

Analysis showed that significant differences existed between the yield obtained from plants inoculated at different stages of growth (Table 17a). Plots inoculated at the pre-flowering stage (R5) had the lowest mean yield of 8.57 kg/plot equivalent to 5288 kg/ha. The maximum yield of 11.5 kg/plot equivalent to 7098.8 kg/ha was obtained on plants inoculated at the primary leaf stage V2. There was a 25.5 % decline between the highest and the lowest yield obtained. Plots infected at the flowering stage (R6) had a total yield of 9.97 kg/ha which is equivalent to 6069.6 kg/ha and was 13.6 % lower than the highest yield obtained.

Table 17a: Analysis of yield (kg/plot) obtained from French beans inoculated at different stages of growth at Naivasha and Kabete

Stage of infection	Kabete	Naivasha
R7	16.67 a	11.00 a
Mancozeb spray	16.60 a	10.46 bcd
V3	15.40 ab	10.60 bc
R6	15.30 ab	9.96 d
V2	14.60 ab	11.50 a
R5	13.56 b	8.57 e
V4	13.43 b	10.10 cd
Mean	15.07	10.33
S.E	0.82	0.18
CV	9.4	3.06

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncan's Multiple Range Test (P = 0.05).

V is for stages of growth in the vegetative phase while R is for stages in the reproductive phase.

V2 is primary leaf stage.

V3 is first trifoliate leaf stage.

V4 is third trifoliate leaf stage.

R5 is pre-flowering stage.

R6 is floweriŋ stage.

R7 is pod formation stage.

Analysis showed that highly significant differences existed between yield obtained at the two sites, but interaction between site and the stage of infection was not significant at either site (Table 17b).

Table 17b: Analysis of yield (kg/plot) obtained from French beans inoculated at different stages of growth on site basis.

Site	Mean yield(kg/rep)
Kabete	15.07
Naivasha	10.33
LSD 0.05	0.70
LSD 0.01	0.94

At Naivasha it was observed that by the second week of harvesting plants inoculated at growth stage V2 had more foliage per plant than plants inoculated at any other stage except the control. With time plots which were inoculated in the later stages of growth lost their foliage while those infected at the early stages of growth (V4, V3 and V2) were recovering from early defoliation. The recovery of foliage could have played a role in the high yields obtained from plants inoculated at growth stages V 2 and V 3.

4.7 EFFECTS OF SPRAYING FRENCH BEANS EARLY AND LATE WITH THREE FOLIAR FUNGICIDES ON THE PROGRESS OF RUST

4.7.1 Kabete site

The disease occurred in mild proportions only and the first infection was observed during the early flowering phase. On all plots infection was uniform with initial severity scores being less than 1% (Table 18). One week after setting in the level of infection increased and most leaves had about 5 % severity. The sudden increase could be attributed to heavy rainfall which had persisted between growth stages R 5 (pre-flowering) and R 6 (flowering). Infection was observed to increase both in severity and incidence (proportion of foliage infected) all through the harvesting period. Due to infection occurring late and close to the harvesting period no foliar sprays were applied.

Table 18: Bean rust severity (%) on French beans sprayed with different fungicides at Kabete.

Treatment	Days after planting							
	21	28	35	42	49	56	63	70
Anvil	0.0	0.0	0.0	0.0	1.2	6.3	10.0	20.0
Baycor	0.0	0.0	0.0	0.0	0.5	4.8	9.6	<20.0
Saprol	0.0	0.0	0.0	0.0	0.5	5.0	12.5	20.0
Unsprayed	0.0	0.0	0.0	0.0	0.5	5.0	10.0	20.0

4.7.2 Naivasha site

(i) Anvil applications

When French beans were sprayed with Anvil at the primary leaf stage there was early and good protection of the first and second trifoliate leaves. The emerging third and subsequent trifoliate leaves were also well protected if the spraying was sustained (Fig 7c, Table 19a). Three consecutive weekly spray applications were made before stopping due to development of phytotoxicity on the plants.

The primary, first and second trifoliate leaves of beans where control started at the pre-flowering phase were well infected by the time sprays were initiated. The primary leaves had already fallen after attaining over 80 % severity (Fig 7b). Infection on the first and second trifoliate leaves was averaging 65 % and was contained at this level when the chemical was sprayed (Fig 7d).

Infection had also set in on the third trifoliate leaves and its spread was limited to 25 % after spraying (Fig 7f). Spraying stopped at the flowering phase. All leaves that emerged after the third trifoliate leaves did not show any infection until the last week of harvesting.

Table 19a: Rust severity (%) on French beans sprayed with Anvil starting at the primary leaf stage and at the early pre-flowering stage.

(i) Spraying at the primary leaf stage

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80++	100[a]				
1st trifoliate		0	0	0	0	5	10	25
3rd trifoliate			0	0	0	5	10	25

(ii) Spraying at the early pre-flowering stage

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80++	100[a]				
1st trifoliate		8	20	65	>65	>65	65	65
3rd trifoliate				25	25	25	20	

[a] After attaining 100 % severity the leaves fell.

(ii) Baycor applications

When applications started at the primary leaf stage the emerging first and third trifoliate leaves had a lower infection (5 %) than on plants where the applications started later (Table 19b). For the first four weeks the primary leaves on plants sprayed early had significantly lower infection than the plants which received applications later.

Although the application rate of the fungicide was doubled after the first spray, no visible signs of phytotoxicity developed on the plants. Four consecutive weekly applications were done before stopping early in the flowering phase because Baycor has a pre-harvest interval requirement of 14 days.

On plots where control started at the pre-flowering stage infection had risen to over 80 % on the primary leaves, 40 % on the first and second trifoliate leaves and was at 25 % on the third trifoliate leaves. Infection increased even after application of the fungicide and was over 80 % by the time harvesting started (Fig 7d, 7f).

Table 19b: Rust severity (%) on French beans sprayed with Baycor starting at the primary leaf stage and at the early pre-flowering stage at Naivasha.

(i) Spraying at the primary leaf stage

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80	100				
1st trifoliate		5	15	25	>25	40	65	>80
3rd trifoliate						5	20	40

(ii) Spraying at the early pre-flowering stage

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80	100				
1st trifoliate		10	25	40	40	53	65	>80
3rd trifoliate			10	25	>25	40	65	>80

(iii) Saprol applications

A 5 % severity score was initially recorded from the first trifoliate leaves of plants sprayed early (Table 19c). This was lower than the 10 % score recorded from plants where control started later. Infection of the third trifoliate leaves was delayed slightly by one week when the plants were sprayed early (Fig 7e, 7f). On this aspect Saprol compared poorly to Baycor which delayed infection of the third trifoliate and subsequent leaves by about three weeks.

Application of Saprol stopped early in the flowering stage because it has a pre-harvest interval requirement of 14 days. This resulted into increased infection which caused a reduction in the yield obtained and also into a build-up of inoculum as the harvesting period came to an end.

Table 19c: Rust severity (%) on French beans sprayed with Saprol starting at the primary leaf stage and at the early pre-flowering stage at Naivasha.

(i) Spraying at the primary leaf stage

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80	100				
1st trifoliate		5	15	25	>25	40	65	>80
3rd trifoliate				5	10	25	40	>65

(ii) Spraying at the early pre-flowering stage

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80	100				
1st trifoliate		10	25	40	53	65	>80	95
3rd trifoliate			8	20	40	65	>80	95

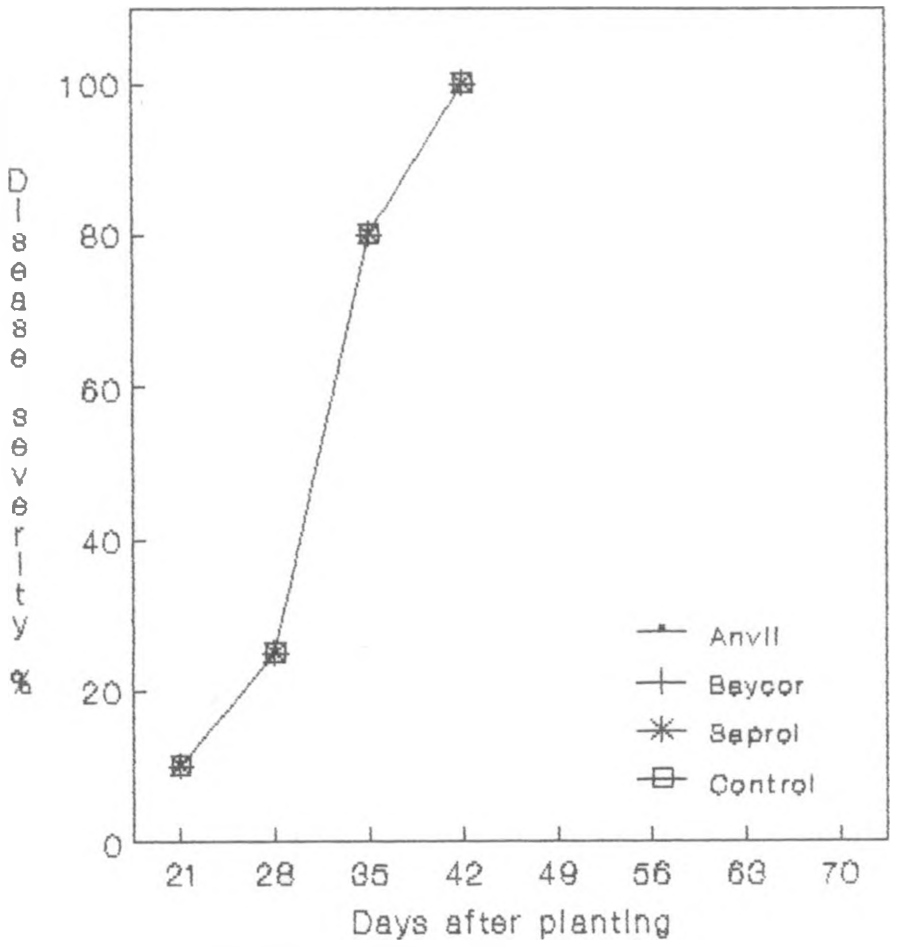


Fig 7a:Disease progress curve comparing data of primary leaves sprayed early with different fungicides at Naivasha

Disease progress was the same and so only one curve is observed for all treatments

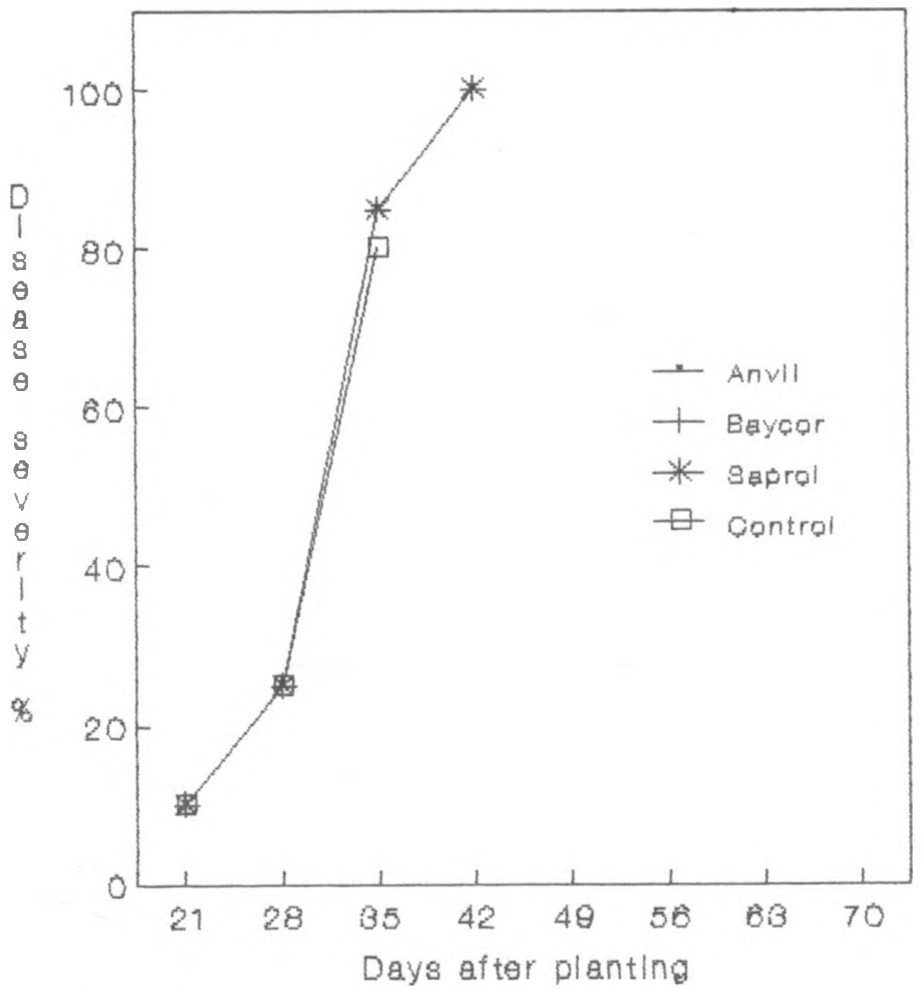


Fig 7b:Disease progress curve for data from primary leaves sprayed late with different fungicides at Naivasha.

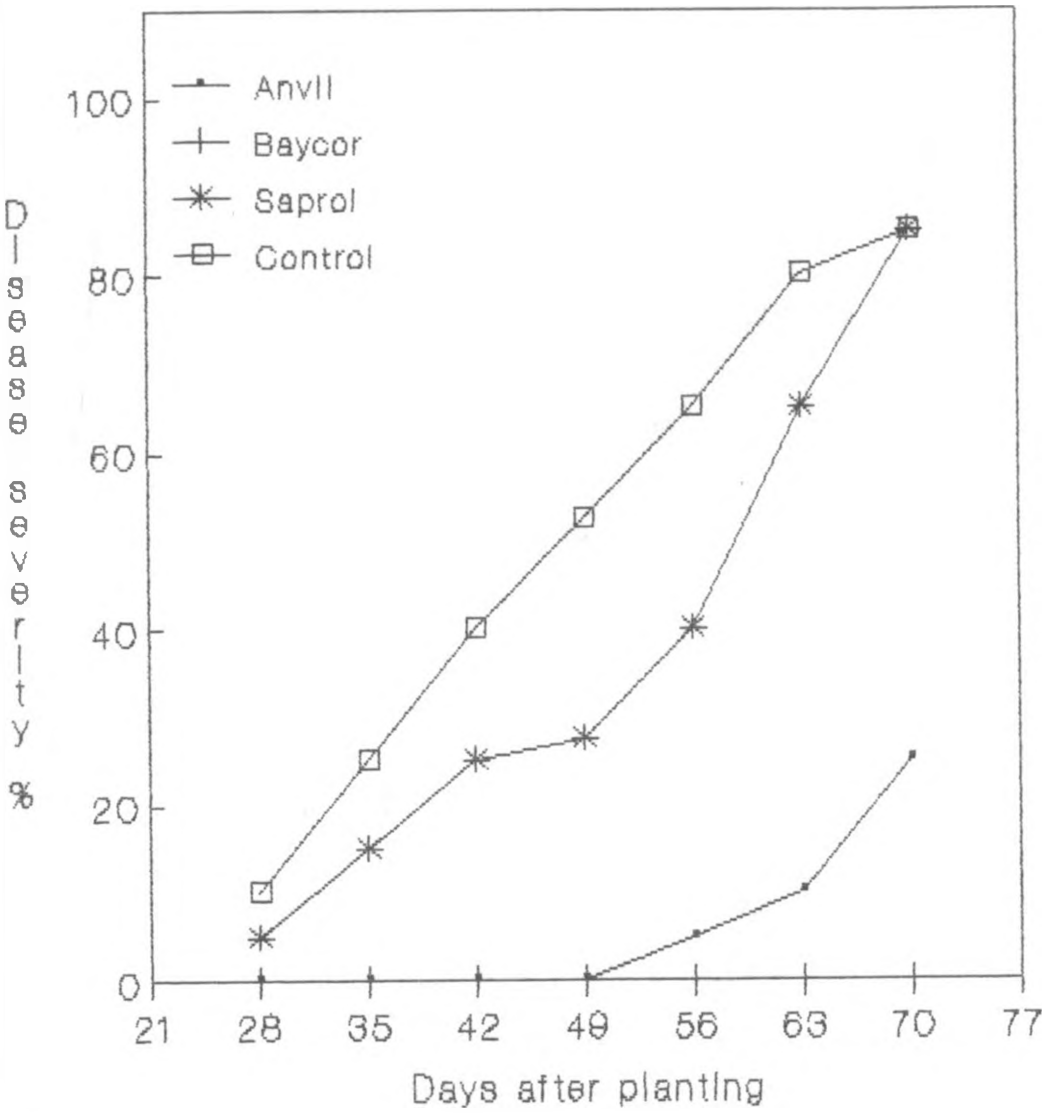


Fig 7c:Disease progress curves of data from 1 st trifoliates sprayed early with different fungicides at Naivasha.

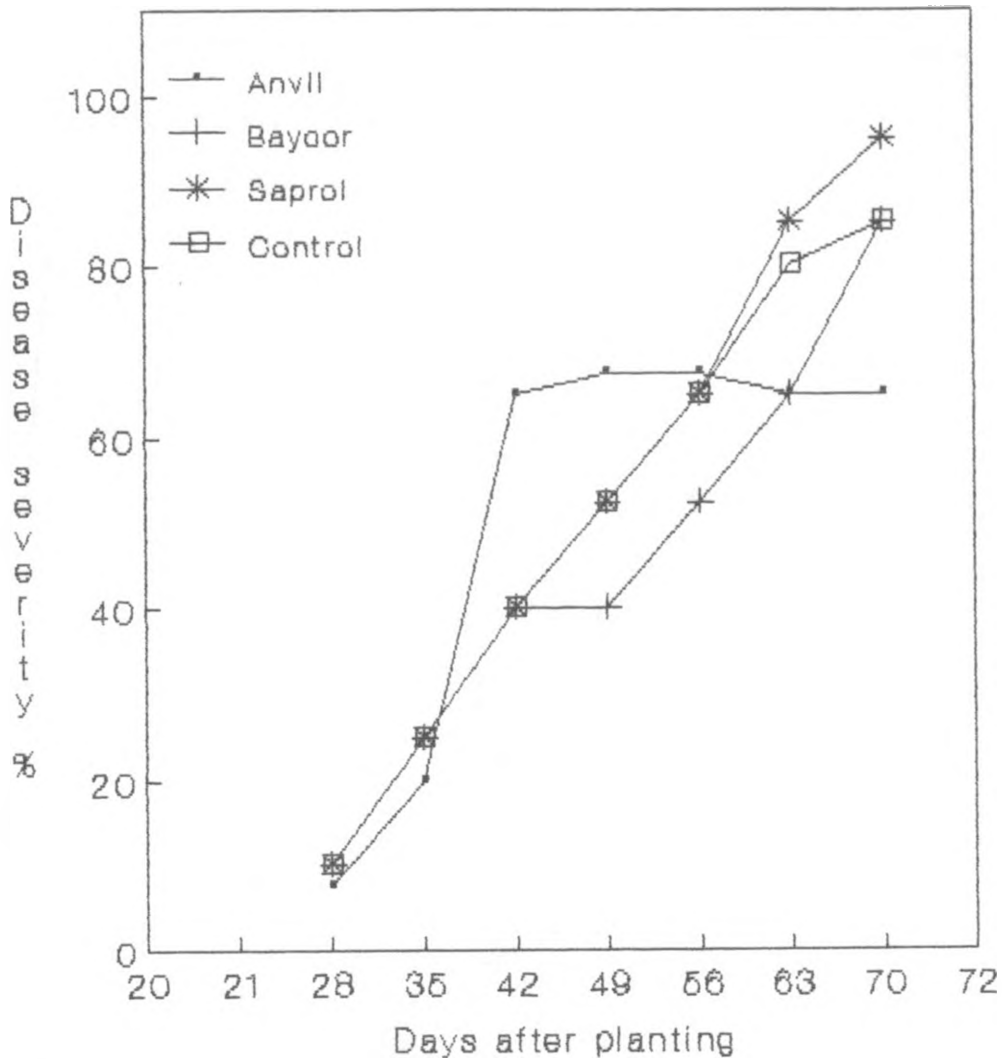


Fig 7d:Disease progress curves of data from first trifoliates sprayed with different fungicides at Naivasha.

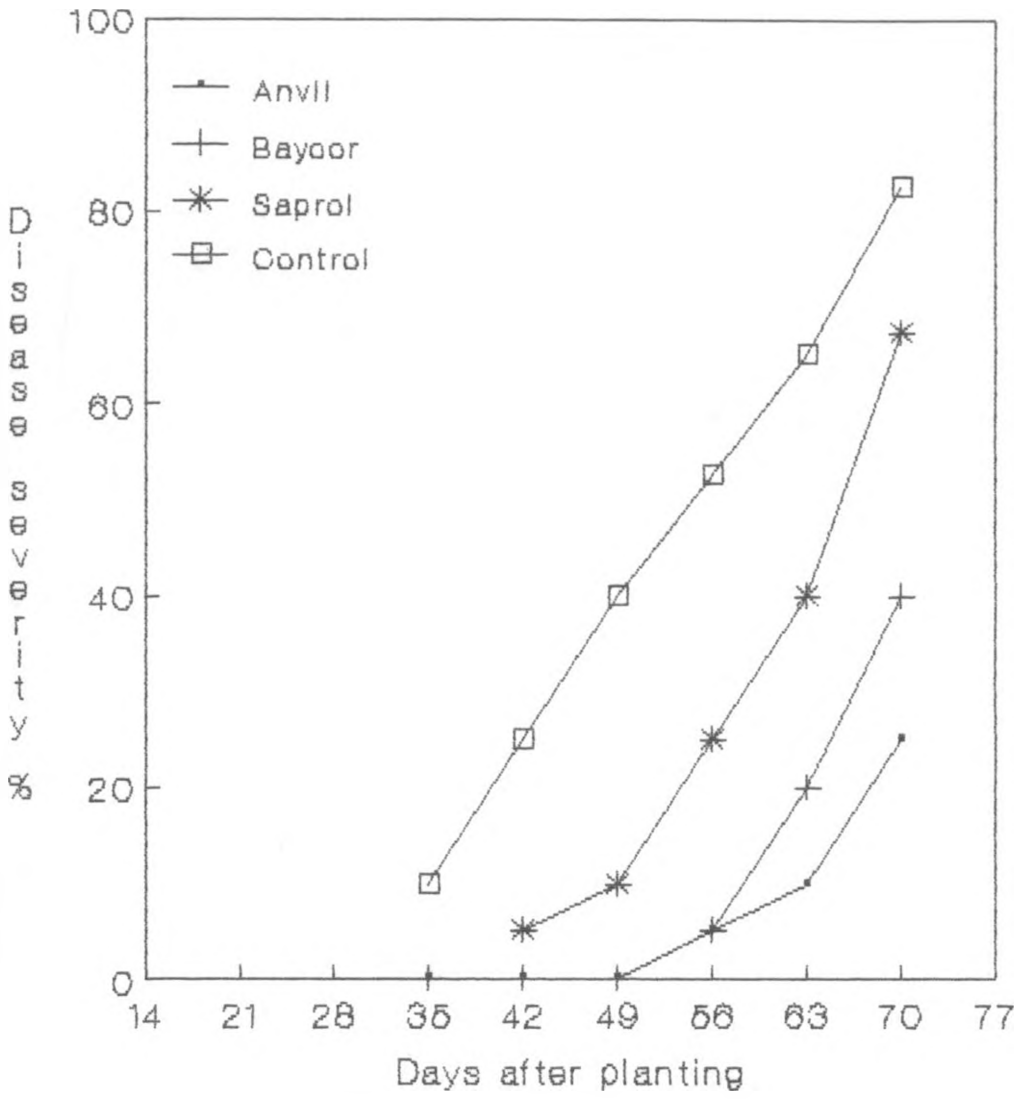


Fig 7e:Disease progress curves of data from 3 rd trifoliates sprayed early with different fungicides at Naivasha.

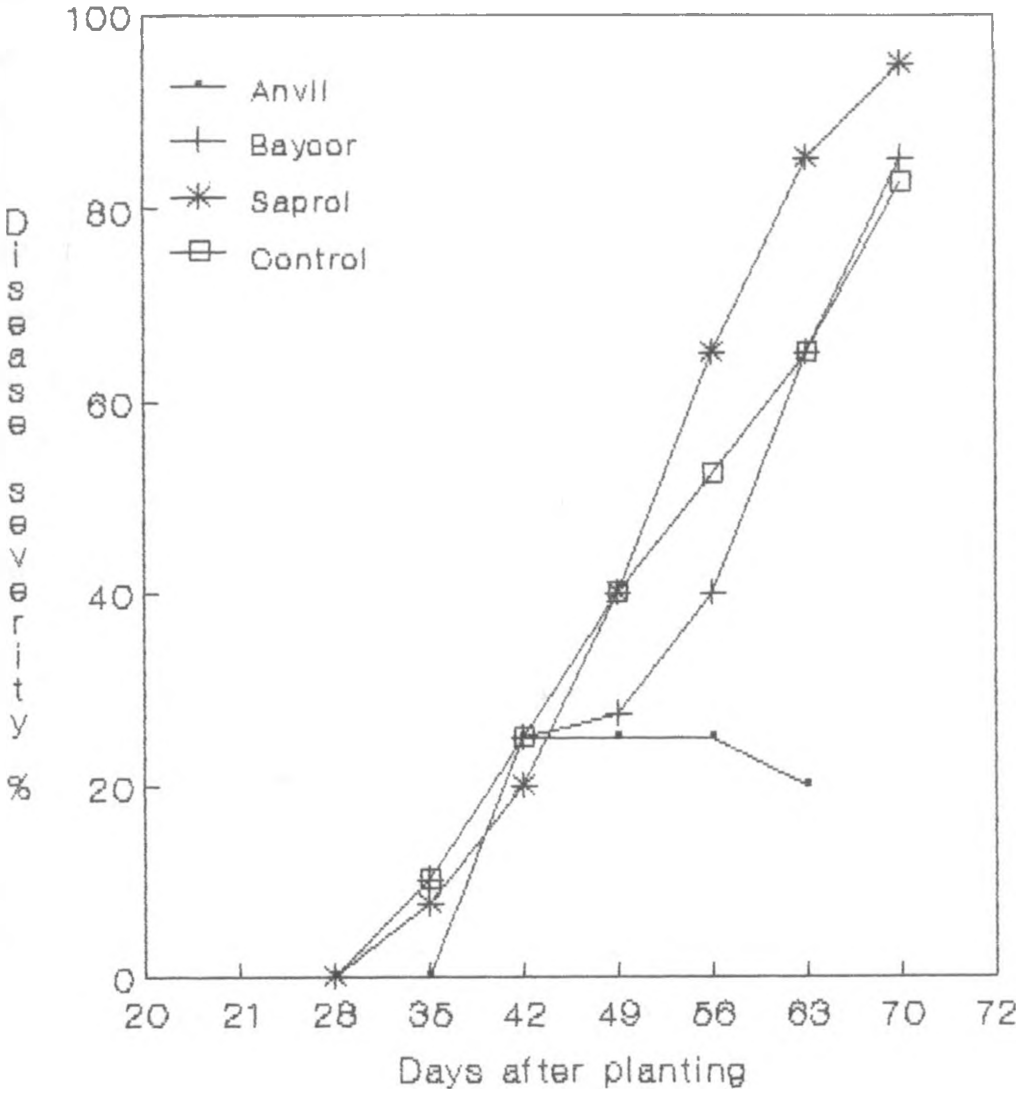


Fig 7f:Disease progress curves of data from third trifoliates sprayed early with different fungicides at Naivasha.

4.8 AREA UNDER DISEASE PROGRESS CURVES (AUDPC's)

Analysis showed that disease progress was the same for all treatments regardless of the time of application and so AUDPC was the same (Table 19d). On the first trifoliate leaves AUDPC value for plots where Anvil was applied early were significantly lower ($P = 0.05$) than for the plots treated with other chemicals. This was due to Anvil's good systemic activity against disease as compared to the other products. The AUDPC value of 22.3 units on the first trifoliate leaves of plots where control started later was much higher than the 1.88 units for plots sprayed early. There was ro significant difference in AUDPC values of plots treated early with Saprol and Baycor but both differed significantly when applications started late.

On the third trifoliate leaves there were significant differences between the plots sprayed early and those sprayed late with all three fungicides. Anvil had the least AUDPC which reflected its good systemic activity against rust.

The AUDPC parameter may not be very good in measuring the extent of disease in the field. For example, on plots sprayed with Baycor and Saprol early the low AUDPC value on the third trifoliate leaves is not a fair representation of the actual scene in the field because it does not show that many leaves were infected unlike in plots sprayed with Anvil where only a few leaves were infected.

Table 19d: Analysis of Area Under Disease Progress Curves (AUDPC) for data recorded from the first and the third trifoliate leaves of French beans where control of rust using Anvil, Baycor and Saprol started at the primary leaf stage and at the early pre-flowering stage at Naivasha.

Treatment	First trifoliate		Third trifoliate	
	Primary	Pre-flower	Primary	Pre-flower
Unsprayed	21.7 a	21.7 a	17.3 a	17.3 b
Saprol	15.3 b	22.2 a	7.8 b	18.2 a
Baycor	15.3 b	18.8 b	2.9 c	14.4 c
Anvil	1.9 c	22.3 a	1.9 d	5.2 d
Mean	13.5	21.2	7.5	13.8
CV	0.8	0.8	2.0	1.1
SE	0.2	0.4	0.3	0.3

Any two means followed by different letters of the alphabet along the columns are significantly different according to Duncans Multiple Range Test (P = 0.05)

4.9 BACTERIAL CELL SUSPENSION SPRAY

The bacterial cell suspensions were applied before infection set in. Suspensions of Isolate CA 5 considerably reduced the amount of infection obtained in the first one week. However, this effect was offset, especially after irrigation when leaves were exposed to infection (Table 19e). On other occasions it was not possible to maintain protection of the plant by the bacterial cell suspensions because they were washed off by unpredictable rain soon after application leaving the plant exposed.

Disease progress on the unprotected control plots and on the plants sprayed with bacterial cell suspensions was the same. Infection on these plants was very severe and resulted in falling off of the first, second and third trifoliate leaves soon after harvesting started. Disease progress curves for the data recorded are shown in Fig 8.

Table 19e: Rust severity (%) on French beans treated with cell suspensions of *Bacillus* sp isolates CA 1 and CA 5 applied at weekly intervals starting at the primary leaf stage and on French beans which did not receive any sprays at Naivasha.

(i) Unsprayed plants

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80					
1st trifoliate		10	25	40	53	65	80	80++
3rd trifoliate			10	25	40	53	65	80++

(ii) Cell suspension sprays

Leaf scored	Days after planting							
	21	28	35	42	49	56	63	70
Primary	10	25	80					
1st trifoliate		10	25	40	53	65	80	80++
3rd trifoliate			10	25	40	53	80	80++

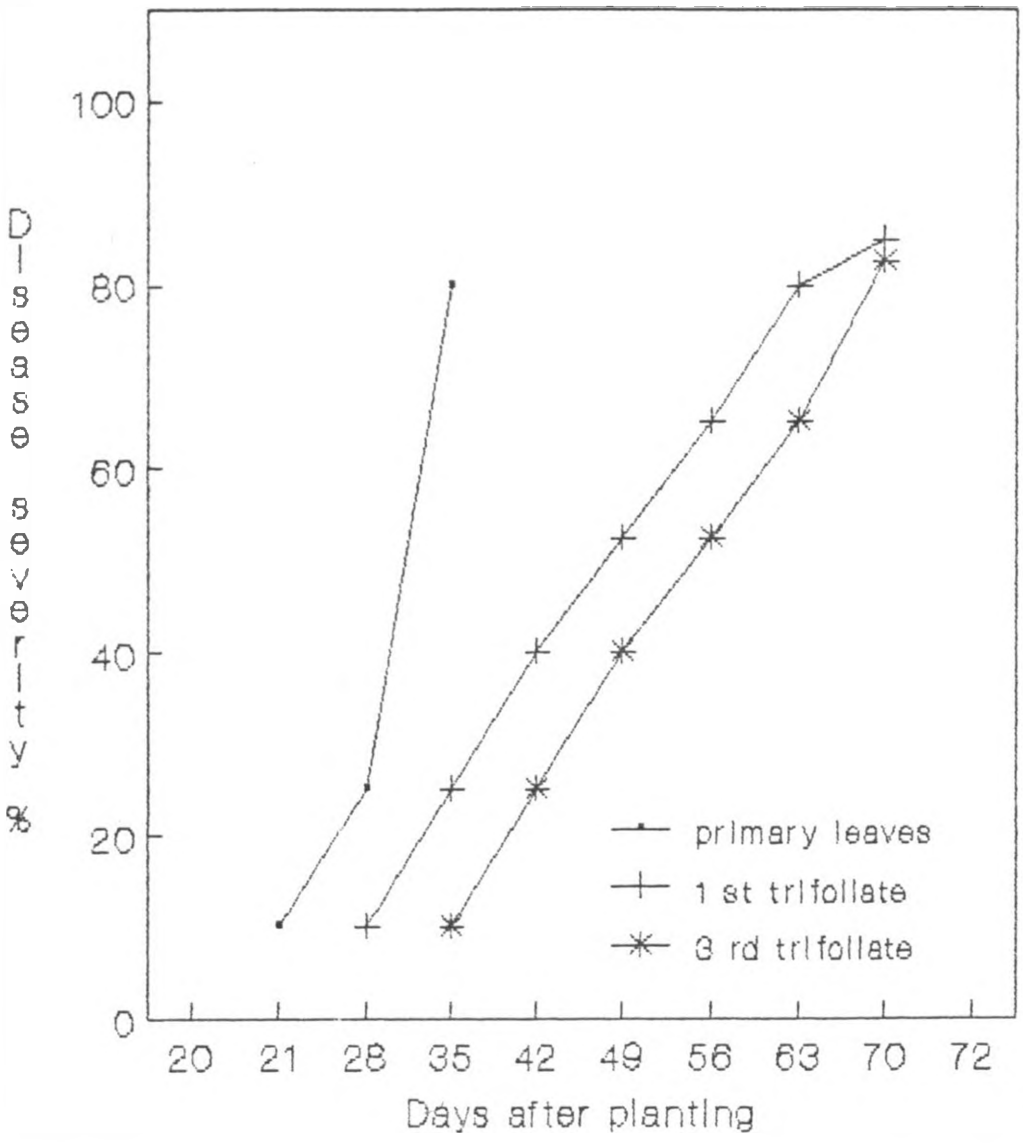


Fig 8: Disease progress curves for data from the untreated control plots at Naivasha.

4.10 YIELD ANALYSIS

Naivasha site

Plots where bean rust was controlled with Anvil starting at the pre-flowering stage had the highest mean yield of 12.3 kg/plot which is equivalent to 10250 kg/ha (Table 20). This was significantly different (P =0.05) from yield of all other treatments. Plots where rust was controlled with Anvil and Baycor at the primary leaf stage had the second highest yield of 10.2 and 10.5 kg/plot respectively (8500 and 8750 kg/ha respectively).

Plots sprayed early with Saprol had 9.2 kg/plot and were better than those sprayed with Baycor or Saprol at the later stages. The control plots had a mean of 8.1 kg/plot and were equal to the plots sprayed with suspensions of *Bacillus* isolate CA 5 but were not significantly better than isolate CA 1 plots with a mean of 7.9 kg/plot (equivalent to 6583 kg/ha).

Kabete site

At the Kabete site the yield obtained did not differ significantly. No infection had been observed. The average yield obtained was 10.5 kg/ replicate plot.

Table 20: Analysis of yield obtained from French beans where control of rust using Anvil, Baycor and Saprol started at the primary leaf stage and at the early pre-flowering stage at Naivasha.

Treatment	Mean yield (kg/rep)
Anvil pre-flowering	12.3 a
Baycor primary leaf	10.5 b
Anvil primary leaf	10.2 b
Saprol primary leaf	9.2 c
Baycor pre-flowering	8.7 d
Saprol pre-flowering	8.7 d
Unsprayed plots	8.1 e
Isolate CA 5 suspensions	8.1 e
Isolate CA 1 suspensions	7.9 e

Any two means followed by different letters of the alphabet along the column are significantly different according to Duncans Multiple Range Test (P = 0.05).

CHAPTER 5: DISCUSSION

BACTERIAL CELL SUSPENSIONS

Results from the study of bacterial cultures of *Bacillus* sp on the control of bean rust demonstrate that the cultures are able to inhibit *Uromyces appendiculatus* from causing rust on beans. The inhibitory effects of *Bacillus sp.* on rust development was shown to be due to its effect on spore germination.

Two isolates CA 1 and CA 5 evaluated in this work suppressed spore germination by more than 80 % by the fourth day of growth. Activity of the cells and their metabolites was found to be optimal after 5 - 6 days of incubation. This was in agreement with seven days optimum period reported by Baker *et al*, (1983,1985). Baker *et al* (1985), monitored cultures over a 10 day period and found that during the log phase growth colony forming units increased to 1 x 10^7 cfu /ml after 20 hr. This figure is considerably lower than the 1 x 10^{12} cfu/ml obtained in this study.

After 4 days in the stationery phase Baker (1985) reported a fluctuation in the number of colony forming units. In this study it was observed that cfu /ml started declining in Isolate CA 5 after day 6 of growth while it was still increasing in Isolate CA 1. Although decline in the number of cfu/ml started earlier in isolate CA 5 than in isolate CA 1 cell suspensions of the former had a more significant effect on spore germination and suppressed rust infection on beans by 97.3 %.

It is known that in its own generation time, a bacterial cell always grows from one standard size to just double the size and then divides (Dean and Cyril, 1966). It thus can be argued

that Isolate CA 5 was more active against bean rust either due to the active metabolites produced being more than for isolate CA 1 or the metabolites had higher activity.

In this study cell suspensions subjected to autoclaving for 15 minutes reduced the mean number of rust pustules by 68 and 81.3 % for isolates CA 1 and CA 5 respectively. This was an average of 16 % lower than the un-autoclaved suspensions for both isolates. This finding was in contradiction to Stavely *et al* (1981) who reported dead cells being as good as live ones and even better than culture filtrates. Baker *et al* (1985) found that autoclaved culture filtrates significantly reduced bean rust.

Baker *et al* (1983) found that when cultures were subjected to centrifugation to remove bacterial cells , the rust inhibiting activity remained in the supernatant and could withstand autoclaving for 10 minutes. This observation resulted in their conclusion that the control of bean rust may be due to a preformed component in the culture filtrates rather than the bacterium *per se*. Pusey and Wilson (1984) reported the control of stone fruit brown rot by *B. subtilis* and also suggested that a heat stable antibiotic was interfering with spore germination or early germ tube development.

The fact that autoclaved suspensions of both isolates CA 1 and CA 5 reduced the mean number of pustules by 68 and 81 % respectively indicates that a portion of the inhibitory metabolite was heat stable and thus was not lost on autoclaving.The presence of a heat stable portion in the inhibitory metabolite is important because it eliminates any danger to the environment that may result from application of the

live bacterial cells.

Other studies found that *B. cereus subsp mycoides* controlled brown spot caused by *Altanaria alternata* on tobacco leaves and suggested the involvement of an inhibitory metabolite or a toxic substance that suppressed conidial germination. Morgan (1963) showed that cereal rust of wheat and oats could be controlled by a spray treatment with *B. pumilus* whose inhibitor was found to be heat labile. Autoclaving the bacterial suspensions reduced phytotoxicity on the treated plants in this study. Effects of certain isolates of *B. subtilis* on plant growth have been noted previously. Chang and Kommedahl (1968) reported the stimulation of plant growth after coating kernels with antagonistic micro-organisms.

Autoclaved cell suspensions did not have any significant effect on rust development under field conditions. This was due to the high inoculum pressure and uncontrollable environmental conditions. Overhead sprinklers used for irrigation during the trial were a hindrance to proper evaluation as the suspensions applied were washed off thus exposing the plants to infection. On a few occasions unpredictable rains washed off the suspensions applied thus exposing the plants.

SEED DRESSING FUNGICIDES

Successful control of foliar diseases by seed dressing has been reported in the past. Schmeling and Kulka (1966) have reported absolute control of bean rust on Pinto beans by treating seeds with Plantvax which is an Oxycarboxin compound that is systemic. Powdery mildews of cucurbits caused by *Sphaerotheca*

fuliginea has been controlled by seed treatment with Benomyl (Neergaard,1976). Rowell (1976) controlled wheat leaf rust, *Puccinia recondita* by seed treatment with the systemic fungicide RH - 124 (4n -butyl -1, 2, 4 - triazole). Paulus and Nelson (1977) controlled downy mildew in Broccoli by treating the seed with Ciba Geigy 48988. In this study none of the evaluated chemicals (Anvil, Baycor, Saprol, Raxil and Real) were found to be effective against bean rust.

The suppression of germination observed when seeds were treated with Raxil and Real was also a negative aspect of their use.Although the germination of seeds 24 hr after treatment was high, the plumule took two days longer to emerge from the cotyledonsthan it took in the control seeds. When seed treated with Raxil and real was stored for two weeks only about 50 % germinated and even these delayed for over two days as compared to the control plants.Germination is a plant growth response controlled by the balance of growth inhibitors and promoters which are usually hormones (Weaver,1972).

In seed dormancies arising from presence of germination inhibitors it is accepted that the inhibitor - promoter balance is usually weighed in favour of the inhibitor. Germination inhibition can arise due to endogenous and exogenous factors which cause partial or specific metabolic block. It was evident in this trial that a factor was introduced with the chemicals which reduced or slowed the germination process. Gibberellins are the hormones which stimulate cell division and cell elongation.

In cereal seed germination the embryo produces gibberellins which diffuse into the aleurone layer where they are thought to

be involved in the control of germination (Radley, 1979). Abscisic acid which occurs endogenously is known to inhibit germination. In this study it is probable that Real and Raxil fungicides had a substance that interfered with the functions of hormones involved in germination, specifically gibberellins.

It is probable that the specific factor that inhibited germination was more suppressive when it remained on the stored seeds which were metabolically inactive for a period of two weeks. It is also possible that the substance induced seed rot and decay thereby reducing germination.

Inability of the chemicals to reduce rust infection on the foliage could have been due to several reasons. It could be that the doses recommended by manufacturers and which were used were not effective. Nene (1971) treated wheat seeds with Plantvax at a rate of 0.25 % and showed that the dose was not enough to protect seedlings from rust caused by *Puccinia striformis*. Powelson and Shanor (1966) had reported the ability of Plantvax to prevent wheat seedlings infection by stripe rust. Poor translocation could have been a factor. Most systemic fungicides have an absorption phenomenon that limits both their absorption by roots and translocation due to accumulation in the leaf margins as was found out by Snel and Edgington (1968). They proved by radioautography that after translocation Plantvax and Vitavax accumulated at the tips and margins of leaves of beans and this inhibited further uptake of the chemical. Breakdown after absorption or conversion of the fungicide into other inactive forms could have contributed to the negative results. Thin layer chromatography techniques showed that after

application as seed treatment, Vitavax is absorbed systemically and is found in the original form or its analogue after passage through the plant.

Three of the fungicides evaluated in this study were not known to have any activity against the bean rust pathogen. Raxil 025, Raxil 040 and Real failed to control bean rust when applied as seed dressing. Plants treated with these fungicides had poor germination, poor stem elongation and malformation in leaf development. This phytotoxic effect was an indication of the interference with growth hormones gibberellins in the plants. Gibberellins function to facilitate internode elongation and control the balance between internode growth and leaf development (Devlin and Witham, 1986).

EFFECT OF SEED DRESSING FUNGICIDES ON RUST DEVELOPMENT IN THE GREEN HOUSE

After assessing plants for infection for five weeks the highest infection severity obtained was 10 % which compared well to the 15 % maximum in the field at Kabete but was very low when compared to the more than 95 % obtained at Naivasha. The poor establishment and slow disease progress was due to the high non-conducive day temperatures which averaged 29 °C in the greenhouse. High temperatures have a negative effect on spore germination and on the subsequent infection (Imhoff *et al*,1981;Berger *et al*,1995). Air movement within the greenhouse was minimal and the only active way by which urediospores were dislodged from the established pustules was during watering when inadvertently water would splash on the leaf surfaces. Although

natural spore dissemination still occurred, inoculum could not spread to the upper newly forming leaves or laterally to the leaves at the same level because spore fall obeys the rules of gravity (Yarwood,1961). In the absence of a strong air current or other suitable mechanism to blow the spores about, such natural dissemination was of no epidemiological significance. Unless conditions enhancing spread of bean rust, such as strong winds to spread inoculum, moist cool weather to ensure prolonged leaf wetness and optimal temperatures prevail then it is highly unlikely that realistic data on disease progress under greenhouse conditions can be obtained.

EFFECT OF SEED DRESSING FUNGICIDES ON RUST DEVELOPMENT IN THE FIELD

At Naivasha the severity scores on the first day of evaluation which was seven days after inoculation averaged 5.9 %. This is in agreement with Imhoff *et al* (1982) who observed that as a bean rust epidemic progresses especially from a focus, 1 - 5 % severity is a typical rating for newly infected leaves. Thus the observed score of 5.9 % was favourable for initiation of an epidemic especially in the favourable weather prevailing.

On the day of initial inoculation it rained and the next day was cloudy throughout thus ensuring enhanced leaf wetness for at least 24 hr which was favourable for infection (Robert,1991; Berger *et al*,1995). After inoculation, temperatures throughout the first week averaged 16° C which lies within the optimal range of 8 - 22° C favourable for infection.

The rate of disease increase between week 1 and 2 was

0.0049. This was low when compared to 0.079 between week 2 and 3 which was the highest during the growing season. This high rate of disease increase could have resulted due to the favourable weather. Average temperatures were a minimum of 10.9 °C and maximum 25.7°C. During that week most days were sunny while in the afternoons it rained. At Naivasha it is usual for sunny days to be accompanied by strong winds which are an important factor in spore dissemination. Favourable weather in the afternoons is of epidemiological importance because such periods are followed by low night temperatures which are good for spore germination and subsequent infection. Infection in a rust epidemic is an essential prerequisite which usually takes place between 6 pm and 6 am (Zadoks, 1971).

The first leaves to fall off did so about 23 days after inoculation with urediospores. This observation could not be conclusively related to infection because as Berger *et al* (1995) observed after following infection on a Phaseolus bean for up to 70 days, natural senescence starts 20 days after plant emergence. Yarwood (1961) found that leaflets having about 200 pustules (+40 % severity on the CIAT scale) would be killed within 20 days. In this study it was observed that the first and second trifoliate leaves only fell off after 28 days , while the third trifoliate leaf fell off after 42 days.

While studying *Septoria glycines* Schuh and Adamowicz (1993) found that infection of leaves of wheat led to premature senescence even at low disease severity. They could not determine any disease threshold for early senescence because leaf senescence was influenced by the location of the lesions on the

leaf in addition to disease severity.This observation may differ slightly in the case of bean rust where in addition to disease severity , leaf senescence also depends on how long the leaf has borne infection.

Imhoff *et al* (1982) has reported that initial pustule densities higher than 5 % kills leaves quickly. Such densities, he further noted are rarely found in areas of disease onset but instead occur almost exclusively in areas where disease has been well established for some time.

By the 21 st day after inoculation disease had reached 99 % severity at Naivasha and the primary leaves were falling off. At the same time disease at Kabete had risen to 2 % severity from an initial mean score of 5 pustules per leaf.The average rates of disease progress were 0.3 and 0.005 for Naivasha and Kabete respectively. Weather conditions at Naivasha were more favourable for rust development than at Kabete. At the Naivasha site which is a hot spot for rust there was continuous generation and dispersal of urediospores in the fields and this enhanced the level of infection.

At Kabete the beans did not get high infection even after a vigorous inoculation routine. When rainfall was not adequate at Naivasha the beans were irrigated more regularly but for shorter durations than for Kabete where irrigation was done for prolonged time periods but less regularly. Such a practice has an effect on the spread of rust because more water means prolonged leaf wetness which favours infection.

STAGE OF BEAN INFECTION WITH RUST AND ITS EFFECTS ON YIELDS

Infection of the bean plant after opening of the third trifoliate leaf was found to have a significant reduction on yield. At Naivasha plants inoculated at growth stage V4 (third trifoliate leaf) had the next lowest yield after plants infected at growth stage R5 (pre-flowering). At Kabete the plants inoculated at growth stage V4 had the lowest yield followed by stage R5. Infection of the bean plant at the lower leaf stages (V2 and V3) did not show any marked effects on yields.

Naturally, primary leaves fall off due to natural senescence at 20 days after emergence (Berger *et al*,1995). In this study defoliation was after 23 days. It is also known that the importance of primary leaves in the growth of the bean plant starts diminishing as soon as the trifoliate leaves open. Trifoliate leaves shade the primary leaves from direct sunlight and thus their photosynthetic value is highly reduced. Jones and Kehinde (1971) found that when wheat plants were infected with *Septoria nodorum* at the growth stage 3 yield was not affected in any way and even there appeared to be a stimulatory effect on the number of fertile tillers and number of grains per head which suggested a compensatory mechanism at work. Compensatory growth may occur due to hormonal changes that are brought about by infection.

Daly and Inman (1958) recorded conspicuous changes in auxin levels in Safflower hypocotyl infected by *Puccinia carthami*. Such hypocotyl showed marked elongation 8 - 13 days after inoculation, during the period of intense mycelial development. A good correlation was obtained between growth and auxin content

of the rust infected hypocotyl. Therefore possible stimulatory effects resulting from early infections in rust infected plants maybe linked to hyperauxiny resulting from an interference with the normal auxin metabolism.

Jones and Kehinde (1971) while working with wheat found that the lower leaves may normally act as a reservoir for inoculum which will be available to infect the upper plant parts but if conditions are not favourable for dispersal, then no damage results.

Reduction in yield when French bean plants were infected at growth stages V4 and R5 could be related to the degree of defoliation attained. At the initial stages of harvesting such plots had lost much of their foliage and on the remaining leaves infection had colonized quite a sizable proportion. Shibairo (1988) while working on pigeon peas found that the number of pods per node was distinctly decreased by defoliation. He further found that defoliation led to shorter plants , reduced leaf area and number ,and lower dry matter accumulated in the various parts of the plant. The degree of defoliation is important and only severe defoliations are bound to have a significant effect on the yield obtained. Hammerton (1975) did not find any significant effect on the total number of pods with mild defoliation ,thus suggesting that the leaves removed were photosynthetically ineffective due to aging and/or mutual shading, or that the plants photosynthetic capacity due to improved light penetration exceeded the necessary pod loads. This explanation could serve well in this work where yields obtained from plants inoculated at stage R6 (flowering) did not differ significantly from those

of the control.

In soyabean, removing 17 or 33 % of the leaf area was compensated for by increased light penetration of the canopy so that yield was unaffected (Turnipseed 1972). Although plants inoculated at growth stage V3 lost more leaves than those inoculated at growth stage V2,there was not much difference between the yield obtained for the two treatments. Pandley and Singh (1981) found that the decrease in yield per Pigeon pea plant was not proportional to defoliation. This shows that the plants inoculated at growth stage V3 were able to compensate for the loss of leaf area. Neales and Incoll (1968) have pointed out that in several species partial defoliation can result in an increased rate of photosynthesis of the remaining leaf area. Such compensation may have occurred in the present work.

Jones and Kehinde (1971) found that infection of wheat by *Septoria nodorum* at the growth stage 6 produced good symptoms on the leaves and later on the heads and resulted in reduced grain yields. This stage can be compared well with the pre-flowering and flowering phases in beans. It is at this stage that the plant is very active in synthesis and transport of nutrients for the development of the seed and infection at this stage can cause considerable reduction to yields. Infection of wheat at the later stages resulted in heavy infection of the flag leaf and sheath, the photosynthetic activity of which contribute much to grain filling and thus yield was significantly reduced.

In beans all the trifoliate leaves that form after the development of the third trifoliate leaf (after stage V4) contribute much photosynthetically to the pod formation and

filling. This study confirms the above fact in that bean yield losses were highest when infection occurred at stages V 4 and R5 at Kabete and Naivasha respectively.

CONTROL OF RUST BY FOLIAR APPLICATION OF CHEMICALS

Application of Anvil starting at the early pre-flowering stages of growth resulted in significantly higher yields than applications of Baycor and Saprol starting either at the primary leaf stage or at the early pre-flowering stage.

Ken *et al* (1987) reported effective control of bean rust using Bitertanol (Baycor) applied weekly starting a week after rust was noticed on the beans. Unlike in this work where phytotoxicity was observed when the application rate was doubled, Ken *et al* (1987) did not report any toxicity even though their rates were higher than recommended ones. Most of the fungicides used in management of rust are triazoles which have both protective and curative activity against their target organisms.

Generally ergosterol biosynthesis inhibitor fungicides such as the ones evaluated in this work have been found to be the most effective in rust control. In the case of Baycor and Saprol it is likely that they had only a relatively short duration of effectiveness inside the plants unlike Anvil which controlled rust better. Rowell (1976) reported that RH-124, a systemic triazole, was able to protect wheat against leaf rust better than triarimol, oxycarboxin and Benomyl but it had a relatively short duration of effectiveness inside the wheat plant.Triforine (Saprol) and Hexaconazole (Anvil) have been reported and are recommended amongst the most effective fungicides in rust

management (HCDA,1996).

The observation that all three fungicides were not effective when applied at the recommended rates could have been due to development of resistance to the fungicides by some of the rust pathogen races. It could also have been due to the very high inoculum levels that were present at Naivasha throughout the experiment period.

Although application of the fungicides stopped early at the flowering stage due to the observed phytotoxicity and also due to the pre-harvest interval requirements, Ken *et al* (1987) has reported that even when rust onset is late in crop development, termination of fungicide sprays at flowering is not recommended. It may therefore be necessary to use chemicals with very short preharvest interval requirements if French beans are to be protected effectively against rust using fungicides.

Biotrophic organisms by definition appear to depend on the metabolic activity of their hosts (Friend and Threlfall, 1975). Investigations with powdery mildews and rusts indicate an initial rise in respiratory activity just prior to the development of visible symptom (Wheeler,1975). An effort has been made to link increased Indole Acetic Acid levels to augmented respiratory activity of the rust infected plant. Auxin levels do not influence respiration directly but rather foster energy requiring processes leading to growth. The rise in respiration is soon followed by a fall which may reflect necrosis of the tissues ending in death of the affected organ or the whole plant (Rubin,1963).

In general the rate of photosynthesis in infected plants begins to decline at about the same time that respiration starts to rise. With disease progress , photosynthesis continues to decline until eventually the quantity of CO_2 given off in respiration by the pathogen and the plant exceeds the amount fixed by the plant (Wheeler,1975). It was shown that C-importing rusted leaves had lower rates of photosynthesis than comparable healthy leaves , while exporting non-infected leaves of the same plants had significantly stimulated rates of photosynthesis. A source-sink relationship could be developed from this effect. Reduction in the rates of photosynthesis may be due to partial necrosis of leaf tissue as seen in peas infected with *Mycosphaerella pinodes* (Rubin,1963). In such cases the greater the extent of infection the more is photosynthesis reduced.

In case of powdery mildews the assimilating surface of the leaf becomes covered with a coating of mycelium which reduces the amount of light available for photosynthesis.

Marked inhibition of $^{14}CO_2$ fixation in the light in late stages of disease has been observed in unifoliate bean leaves heavily infected with rust (Wheeler,1975). At the same time photosynthetic activity in non infected leaves on the same plants increased to levels 1.5 to 2.0 times that of controls (Daly,1967). Infection also can result in reduction in the amount of chlorophyll in the leaf (Rubin,1963). Such a reduction has been observed in the leaves of *Pirola rotundifolia* infected with *Chrysomyxa pirolae*. The reduction is gradual and results in complete disappearance of chloroplast in the cells as observed in wheat infected with *Puccinia graminis f. sp tritici*

(Allen,1926). It is probable that partial breakdown of chlorophyll is not the only reason for reduced photosynthesis. The general disorganization of the process of synthesis and translocation, imbalance of the action of individual enzyme systems and disturbances in the water exchange may also play a part.

The degree of inhibition of photosynthesis depends a lot on the stage of development of the disease and on the virulence of infection. In some diseases there is an increase in the amount of chlorophyll in the tissues surrounding the site of infection. This is observed around the site of infection in bean rust and cereals infected with the rust fungi such as *Puccinia coronifera* and *Puccinia graminis* (Rubin,1963).Plants infected with rust or powdery mildews were observed to fix the greatest amount of CO_2 in the dark at the time of sporulation (Wheeler,1975). Moreover fixation was concentrated in the area of sporulating lesions and thus was attributed to the ability of the fungus to fix CO_2. Bean and cereal rust urediospores possess the malic enzyme which catalyses the reversible reaction, *Pyruvate* + CO_2 <-> *malate* , in both directions. This enzyme may be responsible at least in part for increased dark CO_2 fixation in diseased leaves (Mirocha and Rick,1967). Metabolism of nitrogenous compounds is also affected in the infected plants. Wheat leaves affected by leaf rust have a reduced quantity of nitrogen containing substances. In lucerne affected by *Uromyces striatis* nitrogen losses probably occur by activation of the processes of dissimilation of nitrogen and liberation in gaseous form. In oats infected with rust there's reduced content of protein nitrogen as infection

increases (Rubin,1963). Considerable quantities of nitrogen are utilized by the micro organisms themselves and therefore when the infection is strongly developed the content of amino acids in the infected tissues become very low. The composition of amino acids in the diseased tissue is known to change. Chromatographic analysis found that histidine, leucine and asparagine present in healthy plants were absent in plants infected with *Puccinia graminis f. sp tritici*. Kiraly and Farkas (1957) observed a reduction in the activity of glutamic acid decarboxylase in wheat plants infected with rust. Starch metabolism is also affected. The general pattern is an initial decrease followed by a marked increase with heavy accumulations around the margins of lesions (Wheeler,1975). Diseases caused by obligate parasites bring about intensive accumulation of reserve forms of carbohydrate in the affected tissue (Rubin,1963). Individual pustules and entire leaves act as sinks in translocation processes. Yarwood and Jacobson (1955) observed on kidney beans infected with *Uromyces phaseoli* that the zone of active synthesis of starch corresponds to the zone of maximum infection and that the movement of carbohydrates to the zone of infection continued even when the colonies of the parasite fungus were removed from leaf. Carbohydrates which are present in high concentration in rusted and mildewed leaves are typical of fungal storage material (Friend and Threlfall, 1975). From the foregoing it can be seen that the rust pathogen affects yield both quantitatively and qualitatively by various mechanisms. Obviously infections coming earlier are bound to have more effect on the quantity and quality of yield obtained than those coming much later.

CONCLUSION AND RECOMMENDATIONS

This study found that there is potential in using *Bacillus* sp isolates to control rust on French beans. Although performance was poor in the field, good control of rust was obtained under greenhouse conditions.

The chemicals evaluated as seed dressings did not succeed in controlling or reducing the level of infection on plants both under greenhouse and field conditions. Seed dressing therefore can not be recommended as a method of controlling rust in French beans. Where the chemical option is considered in control of rust in French beans, foliar application remains the most feasible method.

Disease was observed to increase at faster rates at Naivasha which is a hot spot for rust than at Kabete. If production of French beans is to take place under conditions similar to those at Naivasha then measures to control rust must be taken for profitable yields to be realized.

Infection of French beans at growth stages V 4 (third trifoliate) and R 5 (pre-flowering) had the most significant reduction on yields. Application of rust control measures can be timed to coincide with these stages. This would help producers cut on costs incurred through use of manpower and chemicals when application is done earlier than necessary.

Evaluation of the effect of three recommended systemic foliar fungicides (Anvil,Baycor,Saprol) on rust showed that none was effective when applied at the rates recommended by the manufacturers. However, the effect of the fungicides on disease progress and on yield obtained was significantly different when

the chemicals were applied at different stages of growth. Anvil was better than Baycor and Saprol especially when applications started at the pre-flowering phase.

From the results of this study it can be recommended that

1. Chemicals used to manage bean rust should be applied starting from the third trifoliate leaf stage (25 days after planting) but not later than the pre-flowering phase especially in areas where rust is prevalent.

2. Anvil was more effective in controlling rust under field conditions than both Baycor and Saprol and so it is recommended for use.

3. Seed dressing with systemic fungicides was not effective in preventing infection of the bean plant by rust and so other methods of application should be considered when chemicals are being used.

4. Although *Bacillus* sp cell suspensions suppressed germination of spores in the laboratory and also suppressed rust development in the greenhouse, they behaved differently under field conditions. Further study should be carried out to determine the full potential of the *Bacillus* sp isolates in controlling bean rust under field conditions.

REFERENCES

Aart,van Schoonven, Martial,A. Pastor Corrales. 1987. Standard systems for the evaluation of bean germplasm. CIAT.

Agrios,G.N., 1988. Plant Pathology. Third Edition, Academic press Inc.

Alexopoulos,C.J and C.W.Mims., 1979. Introductory Mycology, 3rd Edn. John Wiley and Sons NY. pp 1 - 632.

Allen,R.F., 1926. Cytological studies of forms 9, 21 and 27 of *Puccinia graminis tritici* on Khapli Emmer. J. Agric Res 32 : 701 - 725.

Allen,S.J., Brown,J.F., Kochman,J.K., 1983. The effect of leaf age, host growth stage, leaf injury and pollen on infection of sunflower by *Alternalia helianthi* Phytopathology 73 : 886-893.

Anon,1989. The lure of French beans. Kenyan farmer 1: 29 , Kenya.

Baker,J.C., Stavely,J.R., Thomas,C.A., Sasser,M., Mcfall,J.S., 1983. Inhibitory effects of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. Phytopathology 73 : 1148 - 1152.

Baker,J.C., Stavely,J.R., 1984. Effects of different isolates of *B.subtilis* used for biological control of bean rust under field conditions. Phytopathology 74 : 863.

Baker,J.C., Stavely,J.R., Norton Mock, 1985. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. Plant Disease 69 : 770 - 773.

Bazirake,C.B., 1974. Fungicide efficacy studies on bean rust. Tropical Pesticides Research Institute. Miscellanious Report No. 907.

Ball,A.A., Daly,J.M., 1962. Assay and partial purification of germination from uredospores of the beanrust fungus. Phytopathology 52: 261-266.

Berger,R.D., 1981. Comparison of the Gompertz and Logistic equations to describe plant disease progress. Phytopathology 71: 716 - 719.

Cesar Cardona, Marcial,A. Pastor Corrales, 1992. Strategies for management of pests and diseases of snap beans in Latin America. CIAT, 1992.

Chang,I. and Kommedahl,T., 1968. Biological control of seedling blight of corn by coating kernels with antagonistic micro-organisms. Phytopathology 58 : 1395 - 1401.

Cohen,Y., J.Roten, 1970. The relationship of sporulation to photosynthesis in some obligatory and facultative parasites. Phytopathology 60: 1600 - 1604.

Cooke,B.M., Jones,D.G., 1970. The epidemiology of *Septoria tritici* and *S.nodorum* II. Comparative studies of head infection by *S.tritici* and *S.nodorum* on spring wheat. Trans Brit Mycol Soc 54 : 395-404.

Cunningham,L., .973. Longevity of rust spores in liquic nitrogen. Plant Disease Reporter 57 : 793-795

Daly,J.M., Inman,R.E., 1958. Changes in auxin levels in Safflower hypcotyls infected with *Puccinia carthami*. Phytopathology 48 : 91 - 97.

Daly,J.M., 1967. Some metabolic consequnces of infection by obligate parasites. In "Mirocha, C. J. Uritani, I(Eds). The Dynamic role of molecular constituents in plant parasitic intraction. pp 144 - 164. St. Paul. Am. Phytopathol Soc 1967.

Davison,A.D., E.K.Vaughan, 1963b. Longevity of uredospores race 33 of *U. phaseoli* var *phaseoli* in storage. Phytopathology 53 : 736-737.

Dean,A.C.R., Cyril Hinshelwood, 1966. Growth, function and regulation in bacterial cells. Oxford University Press, 1966 p.46.

Deborah,R. Fravel, Harvey,W. Spurr Jnr, 1977. Biocontrol of Tobacco Brown Spot disease by *Bacillus cereus* subsp *mycoides* in a controlled environment.
Phytopathology 67: 930-932.

Devlin,M.R., Witham, H.F., 1986. Plant Physiology, 4th Edition. pp 1- 577. CBS Publishers and Distributors (Delhi, India).

Dickson,J.H., J.R.Crute, 1974. The influence of seedling age and development of infection of lettuce by *Bremia lactucae*. Annal Appl Biol 76 : 49-61.

Friend,J., D.R.Threlfall (Eds), 1975. Biochemical aspects of plant - parasite relationships. pp 117 - 131.

Grubben,G.J.H., 1977. Leguminous vegetables. In Tropical vegetables and their genetic resources (eds) Tindall,H.D.; William,J.T., Royal Tropical Institute, Amsterdam, Netherlands.

Hammerton,J.L., 1975. Effects of defoliation on Pigeon peas (*Cajanus cajan*). Expl. Agric 11: 177 -182.

Hart,P., Saettler,F., 1981. Bean Rust. Co-op Ext. Svc. Michigan State University Extention Bulletin E. 893. 2p.

Hart,L.P., Endo,R.M., 1981. The effect of length of exposure to inoculum, plant age , root development and root wounding on *Fusarium* yellows of culinary. Phytopathology 71 : 77 - 79.

Harter,L.L., Zaumeyer,W.J., 1941. Differentiation of physiologic races of *Uromyces phaseoli* var *typica* on bean. J. Agric Res 62 : 717-731.

Hawthorn,J., 1981. Foundations of food science. Freeman,W.H. and Co. Oxford and San Francisco, pp. 133.

H. C. D. A. 1996, Kenya Export Development Support, Memoradum, 14th March,1995.

Herminia,A. Francisco, 1992. Production differences between lowland and highland snapbean cultivation .CIAT,1992.

Howland,A.K., McCartney,J.C., 1966. East African Bean Rust Studies .EAFRO 32: 208-210.

Imhoff,M.W., Main,C.E., Leonard,K.J., 1981. Effect of temperature, dew period and age of leaves, spores and source of pustules on germinating bean rust uredospores. Phytopathology 71 : 577-583.

Imhoff,W., Leonard,K.J., Main,C.E., 1982. Pattern of bear rust lesion size increase and spore production. Phytopathology 72 : 441 - 446.

Janssen,W., Guy Henry, 1992. Snapbeans in the developing world. CIAT, 1992.

Jones Gareth, Odebunmi Kehinde, 1971. The epidemiology of *Septoria tritici* and *S. nodorum* IV. The effect of inoculation at different growth stages and on different plant parts. Trans Br Mycol Soc 56 : 281-288.

Juan Guillermo Velasquez, Pedro Prada, 1992. Snapbean pests and diseases in Sumapaz , Colombia : Present status and Implications. CIAT, 1992.

Kaplan,L., 1981. What's the origin of the common bean ? Econ. Bot. 35 : 240.

KBS, 1983. Specifications for fresh green beans. Kenya Bureau of Stancards, Kenya.

Ken Ferzonony, Joyce Francis, George,W. Fong, 1987. Strategies for chemical control of snapbean rust in Florida and their compatibility with Canadian residue tolerances. Plant Disease 71 : 639-642.

Kibata,G.N., 1989. User needs for crop protection information in Kenya. In "'Crop Protection Information ". Eds. Harris and Scot.

Kihurani,A.W., 1989. Differention of isolates of *Uromyces appendiculatus* (Pers. ex. Pers) Unger var *appendiculatus* and evaluation of bean cultivars for resistance to bean rust in Kenya. M. Sc Thesis, University of Nairobi, 1989.

Kiraly,Z., Farkas,G.L., 1957. Decrease in glycolic acid oxidase activity of wheat leaves infected with *Puccinia graminis* var *tritici*. Phytopathology 47 : No. 5. 277 - 278.

Mendgen,K., 1979. Micro autoradiograhy studies on host parasite interaction II. The exchange of 3H - lysin between *Uromyces phaseoli* and *Phaseolus vulgaris*. Arch. Microbiol. 123: 127 - 197.

Mence,M.J., Pegg,G.F., 1971. The Biology of *Peronospora viciae* of pea. Factors affecting the susceptibility of plants to local infection and systemic colonisation. Ann Appl Biol 67 : 297-308.

Mengel,K., 1979. The influence of exogenous factors on the quality and chemical composition of vegetables. Acta. Hort 93:133.

Mirocha,C.J., Rich,P.D., 1967. CO2 fixation in the dark as a nutritional factor in parasitism. In " Mirocha,C.J. and Uritani,I. (Eds): The dynamic role of molecular constituents in plant parasite interactions. pp 121 - 143. St. Paul : American Phytopathological Society, 1967.

Morgan,F.L., 1963 Infection inhibition and germ tube lysis of three cereal rusts by *Bacillus pumillus*. Phytopathology 73 : 1346 -1348.

Mulandi,M.A.S., 1988. Fresh and Processed horticulturae exports. The Horticulture Newsletter 1(2) : 5, Kenya.

NES, 1994. National Enviroment Secretariat. Development of digital biodiversity database of Lake Naivasha region-- Interactions between resources utilisation and the biodiversity. Ministry of Environment and Natural Resources, Kenya.

Neals,T.F., Incoll,L.D., 1968. The control of leaf photosynthetic rate by the assimilate concentration in the leaf. A review of the hypothesis. Bot. Rev. 34 : 107 - 124.

Neergaard,P., 1976. In "Advances in Research and Technology of seeds Part 2. (Ed) J.R.Thompson.p 27 -61.

Nene,Y.L., 1971. Fungicides in plant disease control. Mc Millan Education Ltd, London.

Njeru,L.W., 1989. Influence of Nitrogen rates and storage conditions on quality and shelf life of French beans. M.Sc. Thesis, University of Nairobi,1989.

Njeru,R.W., 1988. Etiology and chemical control of *Cercospora* leafspot of Ornamental Bells of Ireland *Mollucela laevis*. M.Sc Thesis pp. 89, University of Nairobi, Kenya.

Njoya,E.W., 1991. Studies on pigeon pea Leafspot; Etiology and effect of plant age and leaf position on susceptibility of pigeon pea, *Cajanus cajan*, to the leafspot pathogen, *Mycovellosiella cajani*. M. Sc. Thesis, pp. 123, University of Nairobi, Kenya.

Nyadat,N.N., Michieka,D.O., 1970. Soils of Kirima Kimwe, Faculty of Agric farm, National Agricultural Laboratories, M. A, Kenya : 1 - 2.

Nyvall,R.F., Haughland,W.A., 1976. The effect of plant age on the severity of peawilt caused by *Fusarium oxysporum* f sp *pisi* race 5. Phytopathology 66 : 1093-1096.

Opio,A.F.N., 1979. Epidemiology and control of cowpea rust caused by *Uromyces phaseoli* var *vignae* in Kenya. M. Sc . Thesis, University of Nairobi, Kenya.

Pandley,R.K., Singh,V.B., 1981. Influence of source and sink size on flower drop and seed yield on pigeon pea. Indian J. Agric Sci 51 : 185 - 188.

Paulus,A.O., Nelson,J., 1977. Systemic fungicides for control of phycomyctes on vegetable crops applied as seed treatment, granular or foliar spray. Proceedings, 1977 British Crop Protection Conference -Pests and Diseases No. 3 pp 929 - 935.

Powelson,R.L., Shanor,G.E., 1966. An effective seed treatment for systemic control of seedling infection of wheat by stipe rust (*Puccinia striiformis*). Plant Disease Reporter 50 : 806 - 807.

Pusey,P.L., Wilson,C.L., 1984. Post harvest biological control of stone fruit brown rot by *B. subtilis*. Plant Disease 68 : 753 -756.

Ralph,J., Helmut,S., 1983. Farm management Handbook Information Vol II, Central Kenya, Ministry of Agric, Kenya.

Rauveni,M.S., Tuzum,S., Cole,J.S., Siegel,M.R., Kui,J., 1986. The effect of plant age and leaf position on susceptibility of tobacco to blue mold caused by *Peronospora tabacina*. Phytopathology 76 : 455-458.

Robert Hall (Ed), 1991. Compedium of Bean diseases. McMillan Education Ltd, London.

Rose,G.J., 1963. Crop protection. Oxford University Press, Oxford.

Rowell,J.B., 1976. Control of leaf rust on spring wheat by seed treatment with 4 -N -butyl -1, 2, 4 -triazole. Phytopathology 66 : 1129 - 1134.

Rubin,B.A., 1963. Biochemistry and Physiology of plant Immunity. pp 84 -140.

Ryall,A.L., Lipton,W.J.,1978. Handling, transportation and storage of fruits and vegetables. 2nd vol A VI. pub. Co. Inc. Westport.

Schmeling,von B., Kulka,M., 1966. Systemic fungicidal activity of 1, 4 Oxathiin derivatives. Science 152 : 659 - 660.

Schuh,W., Adamowicz,A., 1993. Influence of assessment time and modelling approach on the relationship between temperature - leaf wetness periods and disease parameter of *Septoria glycines*. Phytopathology 83 : 941 - 948.

Shands,W.A., Schein,R.D., 1962. Time - temperature interaction of *Uromyces phaseoli* during spore germination and tube elongation.(Abst). Phytopathology 52 : 27.

Shaner,G., Finney,R.E., 1977. The effect of Nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. Phytopathology 67: 1051 - 1056.

Shibairo,I.S., 1988. Effects of sink - source manipulations and water stress on flower and pod abscission, yield and yield components of Pigeon peas *(Cajanus cajan* L. Millsp.) pp 1 - 153. M. Sc Univer. of Nairobi.

Singh,J.P., Musyimi,A.B.K., 1980. Chemical control of bean rust in Kenya. EAFRO (1980) 45 (3) 207 - 209.

Snel,M., Edgington,L.V., 1968. Fungitoxicity, uptake and translocation of Oxathiin systemic fungicides in beans. Phytopathology 58 : 1068 (Abs).

Spencer,D.M., 1980. Parasitism of carnation rust *Uromyces dianthi* by *Verticillium lecanii*. Trans Br Mycol Soc 74 : 191 - 194.

Stavely,J.R., Thomas,C.A., Baker,C.J., Mc Fall,J.S., 1981. Greenhouse control of bean rust with *Bacillus subtilis* (Abst). Phytopathology 71 : 771 .

Stavely,J.R., 1984. Pathogenic specialization in *Uromyces phaseoli* in the US and rust resistance in beans. Plant Disease 68 : 95 - 99.

Thirumalacar,M.J., Murie,J.O'Brien, 1977. Suppression of char coal rot in potatoes with a bacterial antagonist. Plant Disease Reporter 61 : 543 - 546.

Tindall,H.D., 1983. Vegetables in the tropics. Mc millan Education Ltd, Houndmills,Basingstoke, Hampshire, London.

Tripath,S.S., Singh,P.P., 1968. The association of planting density and plant type in French bean. Expt. Agric 22:427.

Turnipseed,S.G.C., 1972. Response of soyabeans to foliage losses in South carolina. Journal of Economic Entomology 65 : 224 - 229.

Venette,J.R., Jones,D.A., 1982, (Abst). Yield losses associated with severity of bean rust, *Uromyces phaseoli*, on Pinto beans, *P.vulgaris*. Phytopathology 72 : 974.

Wamburi,K.K., 1973. Notes on the Kabete station, Faculty of Agriculture, University of Nairobi.

Warren,R.C., King,J.E., Coulhoun,J., 1971. Reaction of potato leaves to infection by *Phytophthora infestans* in relation to position on the plant. Trans Br Mycol Soc 57 : 501 - 514.

Westcott,C., 1950. Plant Disease Handbook. Oxford Univ.Press, Oxford.

Wheeler Harry, 1975. Plant Pathogenesis. Academic press.

Wyllie,T.D., Williams,L.F., 1965. The effects of temperature and leaf age on development of lesions caused by *Peronospora manshurica* on soybeans. Phytopathology 55 : 166 - 170.

Wynn,W.K., 1976. Appressorium formation over stomates by the bear rust fungus. Response to a surface contact stimulus. Phytopath. 66: 136 - 146.

Yarwod,C.E., Jacobson,L., 1955. Accumulation of chemicals in disease areas of leaves. Phytopathology 45 : 43 - 48.

Yarwood,C.E., 1961. Urediospore production by *Uromyces phaseoli* Phytopathology 51 : 22 - 27.

Zadocks,J.C., 1971. Systems analysis and the dynamics of epicemics. Phytopathology 61 : 600 - 610.

APPENDIX 1:

(i) MEDIA COMPOSITION

Nutrient Broth No. 2

Beef Extract	10 gms
Balanced Peptone No.1	10 gms
NaCl	5 gms

pH 7.3± 0.2

Biotech Laboratories Ltd, UK

Nutrient Agar

Beef Extract	10 gms
Balanced Peptone No.1	10 gms
NaCl	5 gms
Agar	15 gms

pH 7.3 ± 0.2

Biotech Laboratoried , UK

(ii) CHEMICALS USED

Trade name: **Dimethoate**

Common name: Dimethoate 40 Ec

Chemical name: o,o-dimethyl - S -(N- methyl carbamaylmethyl) - phosphorodithioate).

Trade name: **Baycor**

Common name: Bitertanol

Trade name: **Anvil**

Chemical name: Hexaconazole

Trade name: **Daconil**

Common name: Chrolothalonil

Chemical name: Tetra chroloisophthalonitrile 75 %

Inert ingredients 25 %

Empirical Formula $C_8 Cl_4 N_2$

Trade name: **Dithane M -45**

Common name: Mancozeb

Chemical name: ethylene bis (dithiocarbamato) manganese

Formula: $C_4 H_6 N_2 S_4$ 62 %

Inert ingredients 20 %

Trade name: **Saprol**

Common name: Triforine

Chemical name: -1, 4 - di -(2,2,2 -trichrolo - 1 -
formamidoethyl) - piperazine

Formula: $C_{10} H_{14} Cl_6 N_4 O_2$

Active ingredients 18.2 %

Inert ingredients 81.8 %

Trade name: **Benlate**

Common name: Benomyl

Chemical name: Methyl 1 - (butylcarbamoyl) - 2 - benzimidazole carbamate

Formula: $C_{14} H_{18} N_4 O_3$

Active ingredients 50 %

Inert ingredients 50 %

Trade name: Real 200 FS

Common name: Tritoconazole (200 gm/l)

Chemical name: 1RS - (E) -5-((\$- chrolophenyl methylene) -2,2 - dimethyl -1 -(1-H-1, 2,4 trizol -1 -ylmethyl - cyclopentan -1 -ol

Formula: C_{17} H_{20} Cl N_3 O

Trade name: Baxil 025 FS

Common name: Tebuconazole (25g /l)_

Trade name: Baxil 040 FS

Common name: Tebuconazole (20 g/l +Triazoxide (20g/l)

Appendix 2: Effect of age of bacterial cell suspensions on spore germination

Source	SS	df	MS	F	P	
Main Effects						
age	7315.6666667	4	1828.9166667	358.61111111	.0000	***
isol	104.53333333	1	104.53333333	20.496732026	.0002	***
Interaction						
age x isol	28.466666667	4	7.1166666667	1.3954248366	.2715	ns
Error	102	20	5.1			
Total	7550.6666667	2				

Appendix 3: Effect of bacterial cell suspensions on rust development in the green house

Source	SS	df	MS	F	P	
Main Effects						
TREA	856.47222222	5	171.29444444	23.735950731	.0000	***
Error	216.5	30	7.2166666667			
Total	1072.9722222	35				

Appendix 4: Effect of seed dressing on seed germination after storage

Source	SS	df	MS	F	P	
Main Effects						
chem	699.0952381	6	116.51587302	13.298007246	.0000	***
time	84.198412698	1	84.198412698	9.6096014493	.0026	**
rate	6078.3968254	2	3039.1984127	346.86503623	.0000	***
Interaction						
chem x time	3303.4126984	6	550.56878307	62.836654589	.0000	***
chem x rate	3265.047619	12	272.08730159	31.053442029	.0000	***
time x rate	391.44444444	2	195.72222222	22.337862319	.0000	***
chem x time x rate	3906.4444444	12	325.53703704	37.153683575	.0000	***
Error	736	84	8.7619047619			
Total	18464.039683	125				

Appendix 5: Effect of seed dressing on rust development in the green house

Source	SS	df	MS	F	P
Main Effects					
CHEM	217.02787302	6	36.171312169	19.200180869	.0000 ***
TIME	236.54488889	4	59.136222222	31.390239792	.0000 ***
RATE	2080.5857143	2	1040.2928571	552.20034376	.0000 ***
Interaction					
CHEM x TIME	184.70133333	24	7.6958888889	4.0850732184	.0000 ***
CHEM x RATE	145.2471746	12	12.103931217	6.4249167271	.0000 ***
TIME x RATE	285.48063492	8	35.685079365	18.94208247	.0000 ***
CHEM x TIME x RATE	958.33980952	48	19.965412698	10.597888546	.0000 ***
Error	395.62	210	1.8839047619		
Total	4503.5474286	314			

Appendix 6: Yield analysis; seed dressing trial, Kabete

Source	SS	df	MS	F	P
Blocks	16.5175	2	8.25875	2.14829	.1545 ns
Main Effects					
chem	59.9716	7	8.734	2.22006	.0966 ns
Error	54.0158	14	3.855		
Total	130.505	23			

Appendix 7: Yield analysis ; seed dressing trials, Naivasha

Source	SS	df	MS	F	P
Blocks	4.3308333333	2	2.1904166667	2.2583000921	.1412 ns
Main Effects					
chem	28.393333333	7	4.0561904762	4.1818962872	.0110 *
Error	13.579166667	14	0.9699404762		
Total	46.353333333	23			

Appendix 8: Yield analysis; seed dressing trial, combined sites, Kabete and Naivasha

Source	SS	df	MS	F	P
Blocks	17.092916667	2	8.5464583333	3.5909279241	.0400 *
Main Effects					
CHEM	64.249166667	7	9.178452381	3.856470092	.0042 **
SITE	973.80083333	1	973.80083333	409.1576263	.0000 ***
Interaction					
CHEM x SITE	24.115833333	7	3.4451190476	1.4475205644	.2237 ns
Error	71.400416667	30	2.3800138889		
Total	1150.6591667	47			

Appendix 9: Yield analysis; stages of infection, Kabete site

Source	SS	df	MS	F	P
Blocks	10.28	2	5.14	2.53	ns
Stage	30.619	6	5.10	2.51	ns
Error	24.355	12	2.02		
Total	65.258	20			

Appendix 10: Yield analysis; stages of infection, Naivasha site

Source	SS	df	MS	F	P
Blocks	0.2295	2	0.1147	1.0616	ns
Stage	15.7314	6	2.6219	24.25	***
Error	1.297	12	0.10		
Total	17.258	20			

Appendix 11:Yield analysis; stages of infection, bothsites combined

Source	SS	df	MS	F	P
Blocks	4.7222619048	2	2.3611309524	1.9524191747	.1622 ns
Main Effects					
STAG	34.435357143	6	5.7392261905	4.745766113	.0022 **
SITE	236.90625	1	236.90625	195.89777714	.0000 ***
Interaction					
STAG x SITE	11.915833333	6	1.9859722222	1.6422004223	.1756 ns
Error	31.442738095	26	1.2093360806		
Total	319.42244048	41			

Appendix 12: Yield analysis,early and lat foliar sprays, Kabete site

Source	SS	df	MS	F	P
Chemical	7.224	6	1.204	1.21	ns
Error	15.02	14	1.07		
Total	22.25	20			

Appendix 13: Yield analysis, early and late foliar sprays, Naivasha site

Source	SS	df	MS	F	P
Chemical	50.46	8	6.30	1.03	***
Error	1.1	18	0.0611		
Total	51.56	26			

Appendix 14: Analysis of Area Under Disease Progress Curves (AUDPC); first trifoliate leaves sprayed early at Naivasha

Source	SS	df	MS	F	P
Chemical	626.44	3	208.8	4277	***
Error	0.39	8	0.0488		
Total	626.8	11			

Appendix 15: Analysis of AUDPC; first trifoliate leaves sprayed late at Naivasha

Source	SS	df	MS	F	P
Chemical	24.89	3	8.29	64	***
Error	1.037	8	0.1296		
Total	25.933	11			

Appendix 16: Analysis of AUDPC; third trifoliate leaves sprayed early at Naivasha.

Source	SS	df	MS	F	P
Chemical	444.289	3	148	1711.76	***
Error	0.692	8	0.0865		
Total	444.98	11			

Appendix 17: Analysis of AUDPC; third trifoliate leaves sprayed late

Source	SS	df	MS	F	P
Chemical	319.75	3	106.58	1232	***
Error	0.691	8	0.086		
Total	320.44	11			