SEED-BORNE PATHOGENS AND THE BEAN WEEVIL (*Acanthoscelides obtectus*) IN BEAN (*Phaseolus vulgaris*) SEED AND THEIR EFFECT ON SEED GERMINATION AND VIGOUR

by

NAZARETH A. SIYOUM
(B.Sc. Plant Science)

SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY

DISCIPLINE OF PLANT PATHOLOGY

in the

SCHOOL OF APPLIED ENVIRONMENTAL SCIENCE
FACULTY OF SCIENCE AND AGRICULTURE
UNIVERSITY OF NATAL
PIETERMARITZBURG
SOUTH AFRICA

DECEMBER 2003

ABSTRACT

Two semi-selective media, MT and MSP, were compared for their ability to recover cells of the bacteria *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Pseudomonas syringae* pv. *phaseolicola* (Psp), the causal organisms of bean common and halo blights respectively. Isolates Sx70 and Sp75 (for Xap and Psp respectively) were plated on the media by dilution plating. Greater number of Xap colonies on MT than on MSP was obtained, but the number of Psp colonies was not significantly different on the two media. Four media, XCP1, MT, MT_{new}, and TSM were also compared for the number of contaminants and Xap colonies grown on them, using data obtained from ISTA/ISHI Comparative test for *Xap* (2002), which was conducted at the Agricultural Research Council (ARC) in Roodeplaat, north of Pretoria. In most cases, the number of colonies was similar in XCP1, MT, MT_{new}, but bigger in TSM. The number of contaminants was smaller in MT than in MT_{new}.

Bean plants (*Phaseolus vulgaris*), variety PAN 146, were grown and inoculated with the bacteria Xap and Psp, and two virus isolates of bean common mosaic virus (BCMV). Uninoculated controls were also grown. Germination and vigour tests were conducted on the seeds harvested from these plants. Germination was significantly lower for seeds harvested from Psp inoculated plants, while seed vigour was reduced both for seeds harvested from Psp and Xap inoculated plants. Neither germination nor vigour was affected for seeds from BCMV inoculated plants. Late and early matured seeds from the BCMV inoculated plants were compared for the virus transmission and emergence. There was greater virus transmission and reduced emergence in the late matured seeds than in the early matured ones.

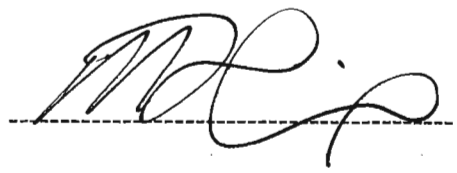
Seeds harvested from Psp and BCMV inoculated plants and uninoculated controls were unexpectedly found to be infested with the bean weevil (*Acanthoscelides obtectus* (Say)). The BCMV infected batch of seeds appeared to repel weevil attack, whereas 25% of the uninfected batch of seeds were attacked.

DECLARATION

I hereby declare that this work is, unless otherwise stated, the result of my own research completed in the School of Applied Environmental Science (Plant Pathology), Faculty of Science and Agriculture, University of Natal.

N.A. Siyoum

September 2003.

Approved by 

Prof. M.D. Laing

(Supervisor)

DEDICATION

I dedicate this thesis to my mother,

Senbetu Tesfe.

ACKNOWLEDGEMENTS

I would like to thank the following people, without whom this thesis would have been impossible.

My Supervisor, Prof. Mark D. Laing, for his sound advice, support, patience and great encouragement without which I would not have succeeded.

My co-supervisor Dr Albert Modi, who taught me most of what I know about seeds, for his constructive criticism, advice and encouragement.

The Eritrean Human Resource Development (EHRD) for sponsoring and funding my project.

Once again my thanks go to Prof. Mark D. Laing, and the University of Natal for the financial assistance I got to accomplish my degree.

Diane Fowlds, Celeste Hunter and Ingrid Schlosser in the Disciplines of Microbiology and Plant Pathology for their technical assistance, help and advice.

All staff members and fellow students of the disciplines of plant pathology and microbiology who helped and supported me through out my work. Thanks to Dr Gus Gubba for providing the virology related information and advice I needed.

Special thanks for my husband David Mehari, for his love, patience, prayer and spiritual encouragement, and always believing in me through my whole university career.

My family and friends for their prayer and words of encouragement.
Above all I thank GOD Almighty for His blessing.

TABLE OF CONTENTS

INTRODUCTION

Beans are adapted to high areas of the tropics and temperate zones, the humid tropics, the semi-arid tropics and in cold climate regions. They are available in a wide geographic area, which extends from 52° north latitude to 32° south latitude, from sea level up to beyond 3000m.a.s.l. (Schoonhoven and Voysest, 1991). Beans are grown on more than 12 million ha and contribute important food for more than 500 million people in Latin America and Africa (Schwartz and Pastor-Corrales, 1989).

Beans are important sources of nutrient requirements, especially proteins, in balanced diet. Apart from proteins, carbohydrates and mineral nutrients such as calcium and iron, vitamins such as thiamine and niacin are obtained from beans. In areas where consumption is high, beans provide more than half of the dietary protein, up to one-quarter of the energy requirements and over 100% of the recommended dietary allowance (RDA) for iron (Schoonhoven and Voysest, 1991).

Seed is fundamental for the perpetuation of bean plants, as the plant is not vegetatively propagated. Consequently, the seed plays a central role in the spread and transfer of seed-borne pathogens both from place to place, and from season to season. More than 50% of the major bean diseases are reported to be seed-borne (Hampton, 1983). Infected seed serves as a source of primary inoculum for seed-borne diseases. Bean common mosaic virus, common and halo bacterial blights are among the economically important and widespread bean diseases that are seed-borne (Galvez and Morales, 1989; Saettler, 1989; Schwartz, 1989). Apart from spreading with the seed, these microorganisms may also affect the germination and vigour of bean seed, although there is paucity of information in the literatures on this area. It was reported that seeds severely infected with the bacterium of halo blight (*Pseudomonas syringae* pv. *phaseolicola*) germinate at low rate, producing deformed seedlings (Katherman *et al.*, 1980; Weller and Saettler, 1980; Saettler *et al.*, 1981).

As a result, seed health testing becomes necessary to ensure that healthy seeds are used as

planting material to produce the next generation of plants. Several methods are used to detect the seed-borne pathogens from the seed: plating seed soak on semi-selective media, immunoassays and DNA techniques. Each of these techniques has disadvantages and advantages. Target bacteria can be detected at 1-5 cfu/ml of seed-soak water using DNA techniques (Prosen *et al.*, 1991). A concentration of 10^2 cells/ml of seed-soak water can be detected in a semi-selective media while only a minimum concentration of 10^5 cell/ml can be detected using ELISA (Narayanasamy, 2001). DNA techniques are more expensive than the other two methods for routine seed health testing, although more sensitive. ELISA, though faster than the plating assay, may not always be suitable for bacteria as the bacteria continuously mutate and change the specific proteins (antigens) the antibodies are developed for. ELISA is commonly used for virus detection because viruses do not grow on artificial media. Detection using semi-selective media is relatively inexpensive and simple for detection of bacteria as compared to the other techniques, although the sensitivity could be lower than the DNA techniques.

REFERENCES

Galvez, G.E. and Morales, F.J. 1989. Aphid-transmitted viruses. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Hampton, R.O. 1983. Seed-borne viruses in crop germplasm resources: disease dissemination risks and germplasm-reclamation technology. *Seed Science and Technology* 11: 535-546.

Katherman, M.J., Wilkinson, R.E. and Beer, S.V. 1980. Resistance and seed infection in three dry bean cultivars exposed to a halo blight epidemic. *Plant Disease* 64: 857-859.

Narayanasamy, P. 2001. Plant pathogen detection and disease diagnosis, 2nd ed. Marcel Dekker, New York.

Prosen, D., Hatziloukas, E., Panopoulos, N.J. and Schaad, N.W. 1991. Direct detection of the halo blight pathogen *Pseudomonas syringae* pv. *phaseolicola* in bean seeds by DNA amplification. *Phytopathology* 81: 1159.

Saettler, A.W., Stadt, S.J. and Pontius, L.T. 1981. Effects of inoculation time and cultivar on internal infection of bean seed by *Pseudomonas phaseolicola. Journal of Seed Science and Technology* 6: 23-30.

Saettler, A.W. 1989. Common bacterial blight. In H.F. Schwartz, and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Schoonhoven, A.V. and Voysest, O. 1991. Common beans: Research for crop Improvement. Cali, Colombia. pp.1

Schwartz, H.F. 1989. Halo blight. In H.F. Schwartz, and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Schwartz, H.F. and Pastor-Corrales, M.A. 1989. Bean production problems in the tropics. 2nd ed. Cali, Colombia. p.ix.

Weller, D.M. and Saettler, A.W. 1980. Evaluation of seed-borne *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* as primary inocula in bean blights. *Phytopathology*, 70: 148-152.

CHAPTER 1

A LITERATURE REVIEW ON SEED-BORNE DISEASES AND WEEVILS OF BEANS

1.1 INTRODUCTION

1.1.1 Beans

Proteins are an essential part of the human and livestock diet, which are obtained from both plants and animals. Legumes provide a large part of plant proteins. Common bean (*Phaseolus vulgaris* (L.)) is one of the major food legumes that are important worldwide. It is surpassed only by soybean (*Glycine max* (L.) Merr.) and peanut (*Arachis hypogea* (L.)) (Singh, 1999).

Beans are a good source of proteins, giving a complete protein diet when mixed with cereals. Dry beans do not contain cholesterol and are therefore beneficial to human health. Regular intake of beans may help to lower cancer risks (Singh, 1999).

Beans originated in Latin America and were spread all over the world by traders. Currently Africa is the third largest producer preceded by Latin America and North America (FAO, 1990 cited in Spence and Walkey, 1994).

1.1.2 Bean Diseases

Beans are affected by many fungal, bacterial and viral diseases. Bean diseases like anthracnose, common bacterial blight and bean common mosaic can cause extensive or complete crop failure and are important throughout the bean production areas of the world (Hall, 1991).

Several of these important bean diseases are seed-borne. The pathogens over-winter or stay viable in the seed after harvest. Infected seeds give rise to diseased seedlings and plants. Complete infection of plants may result from a certain level of seed infection,

aided by agents like insects (Pastor-Corrales and Tu, 1989) and under favourable conditions to the pathogen, badly infected seeds may result in a complete loss of yield (Zaumeyer and Thomas, 1957, cited Pastor-Corrales and Tu, 1989).

Control of these seed-borne diseases is primarily based on the use of "disease free" seed. Disease free seed production is mostly accomplished in areas that have unfavourable conditions for the development of the pathogen and are free of insect vectors.

Other control methods include quarantine and plant inspection. Seeds are tested for the presence of the important seed-borne diseases when moved to a new area. Actively growing plants are also inspected for symptoms. Plants with the symptoms are discarded or rouged and destroyed.

1.2 DETECTION OF SEED-BORNE PATHOGENS

Seed-borne pathogens are detected using various methods depending on the type of the pathogen, where the pathogen is present in the seed, the objective of the seed health test, etc. Different fungi can be detected using the blotter method. Seeds are incubated on moist blotter paper for some period and evaluated for growth of fungi morphologically. Bacteria can be detected by soaking seeds in sterile water and plating the liquid on selective or semi-selective media. Viruses cannot be detected with media. Viruses can be detected by planting seeds and evaluating seedlings for symptoms and/or by immunoassays and DNA techniques. These methods for virus detection can also be applied for fungi and bacteria.

Pathogens are seated on different positions in the seed. Some are found only on the seed coat, others on cotyledons and embryo. The position of a pathogen in or on seed determines the method to be used.

Detection methods also depend on the purpose of the seed health testing. According to Neergaard (1977) the following purposes for seed health testing are listed:

1.2.1 Testing for Quarantine

This is typically conducted on particularly valuable seed material by growing it in a greenhouse until harvest to exclude dangerous pathogens, but is an expensive procedure. Seeds for export which should be 'substantially free' from a particular pathogen are tested by sufficiently sensitive methods to reveal traces of infection.

1.2.2 Testing for Certification Schemes

The purpose of this test is elimination of pathogens. Selective procedures aiming at detection of maximum levels of pathogens can be used. Indicator tests revealing traces should be considered for viruses. Agar plates for fungi and agar plates combined with seedling-symptom can be suitable for bacteria.

1.2.3 Testing for Evaluation of Planting Value

The aim of the test is to forecast field emergence and to some extent, the health of the mature crop. The tolerance for different pathogens varies greatly, depending also on different conditions.

1.2.4 Testing for Advisability of Seed Treatment

This aims at testing the seed health if seed can be used without treatment; if it can be used after treatment according to prescription or if it is unsuitable for sowing.

1.2.5 Testing Treated Seeds

The health condition of seeds treated by chemicals or other means is checked . This is particularly important in connection with inspection of seeds for export in accordance with quarantine regulations.

1.3 METHODS OF DETECTION

There are various methods of detecting seed-borne pathogens. Commonly used methods

include the following:

1.3.1 The Blotter Paper Method

In this method seed samples are surface sterilized when testing for internally seed-borne pathogen and incubated on moist blotter paper under favourable conditions for the pathogen for several days. Fungal growths are examined under stereomicroscope. This test is qualitative and is suitable for detection of seed-borne fungi.

1.3.2 The Agar Plate Method

a) Plating seeds directly on agar. Selective medium suitable for the desired pathogen is prepared in petri dishes. Seeds are first surface sterilized and incubated on the agar at room temperature for 5-8 days (Neergaard, 1977). Using this method seed-borne fungi are quantitatively and qualitatively detected according to their colony type on the agar media.

b) Plating extracts on agar medium. In this method seed samples are soaked in water for several hours without pre-treatment. Liquid extract is plated on agar medium suitable for the specific pathogen on test. This method is used for quantitative and qualitative detection of seed-borne bacteria according to colony morphology.

1.3.3 Symptom Evaluation Test

Seed samples are planted under controlled environment to avoid external contamination. Seedlings are evaluated for symptom development. Both fungi, bacteria and viruses can be detected qualitatively using this method.

1.3.4 Serological Methods and Polymerase Chain Reaction (PCR)

In most cases suspected colonies obtained from previously tested seed samples or plant materials with the symptom for the specific pathogen are tested using these methods. The serological methods such as ELISA use specific antisera for the test pathogens while the PCR uses specific target nucleic acid of the pathogen. These methods produce qualitative

as well as quantitative results, and are especially good for viruses, but can be applied to fungi and bacteria too.

1.4 SEED GERMINATION AND VIGOUR

Seed germination, according to ISTA, is defined as "the emergence and development of a seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil" (ISTA, 1999).

Seed vigour is defined by ISTA as "the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Seeds which perform well are termed 'high vigour' " (Perry, 1978).

Germination value is basically dependant on the essential structures and does not deal with the level of activity and performance of germination and unfavourable conditions that might be encountered in the field. However vigour value takes these values and conditions into account.

As it is described in Copeland and McDonald (1995), aspects of seed germination performance include: 1) biochemical processes and reactions during germination such as enzyme reactions and respiration activity, 2) rate and uniformity of seed germination and seedling growth, 3) rate and uniformity of seedling emergence and growth in the field, and 4) emergence ability of seedlings under unfavourable environmental conditions. Copeland and McDonald also mentioned factors that influence the level of seed vigour include the genetic constitution of the seed, environment and nutrition of the mother plant, stage of maturity at harvest, seed size, weight and specific gravity, mechanical integrity, aging and pathogens.

A seed reaches its maximum germination potential at a stage when it accumulates maximum dry weight, which is termed physiological maturity and is believed to acquire

highest vigour at this stage (McDonald *et al.*, 2001). Burris *et al.* (1971) demonstrated that large soybean seeds were superior to small seeds in germination and vigour. This indicated that the environment during seed maturation has an indirect effect on seed vigour.

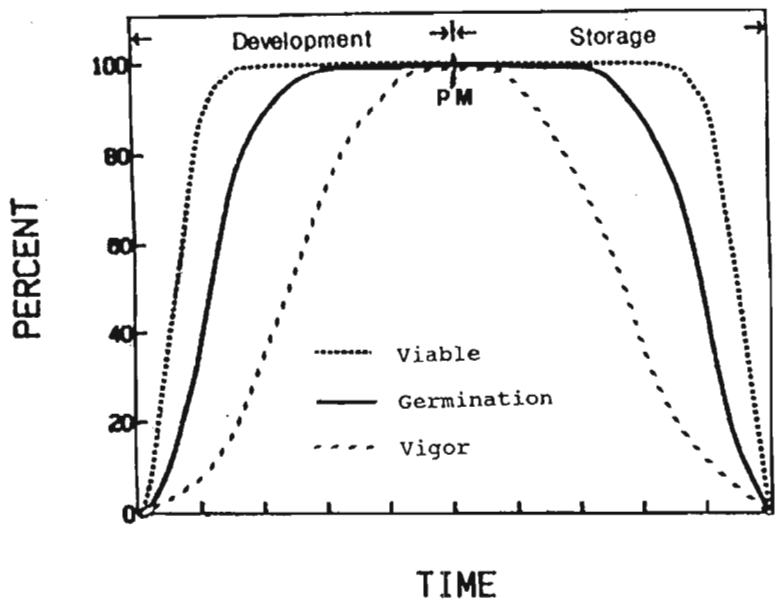


Figure 1. Pattern of increase in seed viability, germination and vigour during seed development prior to physiological maturity and decrease in seed quality during storage. (Graph taken from McDonald *et al.*, 2001)

Seed deterioration begins after physiological maturity while the seed is in storage on the plant and continues after harvest, and is greatly influenced by genetic, production and environmental factors (McDonald *et al.*, 2001). Figure 1 illustrates the process of deterioration, the seeds' physiological quality changes in inverse order of seed development with seed vigour declining first, followed by loss of germination and viability.

Microorganisms are known to cause both positive and negative effects on germination. Campbell (1985) pointed out that beneficial effect of microorganisms on germination

could be in the decay of hard pericarps of some species whose embryo cannot break out of the protective cover or cannot imbibe water through it. Campbell also mentioned that this is one of the reasons for stratifying some seeds. During stratification the embryo may mature or the dormancy may be broken by moderate temperature after chilling or freezing. Further microbial weakening of the seed coat or the pericarp is achieved.

Other microorganisms may have a harmful effect on seed germination and vigour. Wheat seeds infected with *Tilletia indica* that causes Karnal bunt were found to have reduced germination and vigour, especially at higher infection rates (Warham, 1990). Other studies also showed that as Karnal bunt infection increases, the percentage of seed germination decreases; seeds with moderate to severe infections tend to produce a greater percentage of abnormal seedlings (Rai and Singh, 1978; Singh, 1980; Singh and Krishna, 1982, Aujla *et al.*, 1983; Bansal *et al.*, 1984, cited in Warham, 1990).

Campbell (1985) stated that there are a number of minor pathogens of seeds and seedlings that decrease germination and emergence. Fungi, including *Cylindrocarpon destructans, Fusarium sambucinum, and Gliocladium roseum*, were controlled with fungicides, resulting in an increased number of seedlings even though there was no obvious disease in the absence of the fungicide. Campbell mentioned that bacteria, especially Gram-negative ones, may also reduce germination. The reduced germination is thought to be due to competition of the microorganisms with the seed for oxygen.

Vennette (1985) worked on the effect of bacterial pathogens, namely *Pseudomonas syringae* pathovars *phaseolicola* and *syringae* on bean emergence. Emergence was significantly reduced by these pathogens, especially at a high concentration of $3x10^6$ cfu/ml.

Germination is the ability of a seed to develop into a normal seedling under favourable conditions, which requires a simple and direct test. However, seed vigour is not such a simple character of a seed that can be tested directly. Therefore many of the vigour tests involve indirect measurements. Two of the methods used to measure seed vigour are

mentioned here.

1.4.1 Conductivity Test

This test measures the amount of electrolyte leakage from plant tissue and was first developed for seeds of several crop species by Hibbard and Miller (1928) (cited in ISTA, 1995). The integrity of cell membranes, determined by deteriorative biochemical changes and/or physical disruption, can be considered the fundamental cause of differences in seed vigour which are indirectly determined as electrolyte leakage during the conductivity test (Powell, 1988, cited in ISTA, 1995). Low vigour seeds have been shown to possess decreased membrane integrity as a result of storage deterioration and mechanical injury (Copeland and McDonald, 1995). As a result, during imbibition, seeds with poor membrane structure release cytoplasmic solutes that can be detected by a conductivity meter.

1.4.2 Accelerated Aging Test

Accelerated aging was initially developed as a test to estimate the longevity of seed in commercial storage (Delouche and Baskin, 1973) and has been used to predict the life span of a number of different species (ISTA, 1995). They also mention that the test has subsequently evaluated as an indicator of seed vigour in a wide range of crop species and has been successfully related to field emergence and stand establishment. This test incorporates many of the important traits desired in a vigour test by subjecting unimbibed seeds to conditions of high temperature and relative humidity for short periods (Copeland and McDonald, 1995). The seeds are then removed from the stress conditions and placed under optimum germination conditions. The decline in germination following accelerated aging is proportional to the initial physiological potential of the seed (Warham, 1990). High vigour seeds show only small decrease in germination following accelerated aging while low vigour seeds show marked decreases (Delouche and Baskin, 1973).

1.5 SEED-BORNE PATHOGENS AND THE BEAN WEEVIL

1.5.1 Halo Blight of Beans

1.5.1.1 The Pathogen

Halo blight of beans is caused by the bacterium *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young *et al.* (1978) (Schwartz, 1989). The bacteria cells are gram-negative, strictly aerobic, single straight rods, and move by using multitrichous polar flagellae (Schwartz, 1989).

1.5.1.2 Host Range

The pathogen can infect various plant species including the tepary bean (*Phaseolus acutifolius* A. Gray var. *acutifolius*), *Macroptilium bracteatum* (Nees ex Mart.) Marechal et Baudet, scarlet runner bean (*P. coccineus* L.), lima bean (*P. lunatus* L.), *P. polyanthus* Greenman., *P. polystachyus* (L.) B.S.P., common bean (*P. vulgaris* L.), pigeonpea (*Cajanus cajan* (L.) Millsp), hyacinth bean (*Lablab purpureus* (L.) Sweet), soybean (*Glycine max* (L.) Merril), *Vigna angularis* (Willd.) Ohwi et Ohasi, mung bean (*V. radiata* (L.) Wilczek var *radiata*), *Pueraria lobata* (Willd.) Ohwi, and siratro (*Macropitilium atropupureum* (DC.) Urb.) (Zaumeyer and Thomas, 1957; Walker, 1969; CIAT, 1987 cited in Schwartz, 1989).

1.5.1.3 Geographic Distribution and Economic Importance

Halo blight of beans occurs worldwide including in regions of Latin America which have moderate temperatures, the Great Lakes Region of Africa (Rwanda, Brundi, and Zaire), eastern Africa (Costa, 1972; Dubin and Ciampi, 1974; CIAT, 1981; Allen, 1983; cited in Schwartz, 1989), Great Britain (Taylor and Dudley, 1976) and North America (Walker and Patel, 1964).

The disease can cause extensive losses in yield and quality under epidemic conditions (Webster *et al.*, 1983). Researchers showed that halo blight caused a yield loss of 23-43%

(Saettler and Potter, 1970 cited in Schwartz, 1989).

1.5.1.4 Disease Cycle and Epidemiology

The principal source of infection is contaminated seed. Early infection of the plant by the bacterium results in higher seed transmission (Saettler *et al.*, 1981, cited in Schwartz, 1989). When infection is from external sources, wounds or stomata are the means by which the pathogen enters plants during periods of high relative humidity or free moisture (Zaumeyer and Thomas, 1957; Walker and Patel, 1964a; Saettler and Potter, 1970, cited by Schwartz, 1989). Halo blight symptoms develop in 6-10 days in 24-28^{0}C and may be delayed at higher temperatures (Zaumeyer and Thomas, 1957, cited in Schwartz, 1989). Cool weather favours production of the toxin that forms the distinctive halos and systemic yellowish symptom (Harveson, undated). Halo symptoms usually do not develop above 28^{0}C, although small and numerous water-soaked lesions may still be present (Zaumeyer and Thomas, 1957, cited in Schwartz, 1989). Favourable conditions for the pathogen are moderate temperatures, 16-23^{0}C and humid moist conditions while the optimum temperature range is 20-23^{0}C (Davis *et al.*, 2002; Schwartz, 1989).

The bacterium Psp can over-winter in undecayed, infected plant debris (Gubler *et al.*, 1990) or in volunteer beans in the field (Legard and Schwartz, 1987 cited in Schwartz, 1989) until environmental conditions are favourable for infection.

Wind-driven rain, overhead irrigation, equipment, or people and animals can spread halo blight (Dillard and Legard, 1991).

Pods are infected locally or by systemic vascular invasion while seeds are infected by surface contamination from the pod wall or by systematic invasion through the funiculus (Walker, 1950).

1.5.1.5 Symptoms

In the early (3-5) days after infection small water-soaked spots appear on the underside of

the leaves (Gubler *et al.*, 1990; Allen, 1987). Later halo of greenish-yellow, tissue develops around the lesions (Allen, 1987). During severe epidemics the stem and the pods may also became infected and produce the typical greasy spots (Schwartz, 1989). Infected seeds give rise to seedlings with stem girdling and joint rot at nodes (Allen, 1987). Plants grown from infected seed can be stunted, spotted or yellow, deformed which will eventually die (Dennis, 1952).

Seeds developing from infected pods are likely to be spotted, wrinkled or shrivelled, whereas sometimes they may bear no obvious mark of infection and yet give rise to infected seedlings (Dennis, 1952).

1.5.1.6 Seed Infection and Phytosanitary Significance

The pathogen is seed-borne which is found in the inner layer of the seed coat and cotyledons (Taylor *et al.* 1979). Seed surface contamination with Psp also produces infected plants (Guthrie, 1970). The bacterium can survive more than four years in bean seed (Dillard and Legard, 1991).

Infected seed is the main source of primary inoculum (Taylor and Dudley, 1979). Guthrie *et al.* (1965) indicated that low levels of seed infection are capable of initiating heavy field infections causing severe crop loss when environmental conditions are favourable. Even levels as low as a single contaminated seed in 16,000 can cause a severe epidemic under favourable weather conditions (Dillard and Legard, 1991). Bean seeds harvested from field in which the disease had not been detected during the growing season were found to transmit Psp (Grogan and Kimble, 1967).

1.5.1.7 Control

Infected bean tissue on the soil surface and volunteer beans are means of survival for pathogen between growing seasons (Schuster and Coyne, 1976b; Legard and Schwartz, 1987, cited in Schwartz, 1989). Therefore deep-ploughing is likely to reduce initial inoculum pressure (Zaumeyer and Thomas, 1957, cited in Schwartz, 1989). Crop rotation

of about three years is advisable as it is sufficient to dispose off over-wintering organisms (Walker, 1950). This also implies that sanitation of bean straw contributes to reducing initial inoculum.

Disease-free seed produced under conditions unfavourable to organism should be used to reduce the initial inoculum within a field (Zaumeyer and Thomas, 1957, cited in Schwartz, 1989). In South Africa Seed Certification Scheme, disease free dry bean seed is produced through meristem cultural procedures and multiplication in the greenhouse (CBS, 2003).

Chemicals or antibiotics can be used to destroy bacteria present on seed surfaces (Hagedorn, 1967; Rusel, 1975; Zaumeyer and Thomas, 1957, cited in Schwartz, 1989).

Heat treatment of seeds is also used for reducing bacterial infection. For example, the proportion of infected seedlings was reduced by more than 70% by exposing dry seeds to 70^0C for 120 minutes or water soaked seeds to 50^0C for 180 minutes (Belletti and Tamietti, 1982, cited in Schwartz, 1989).

Growing resistant varieties is the best means of preventing heavy loses in commercial bean production (Dennis, 1952).

Schwartz (1989) points out that successful long-term control of Psp requires that bean-production regions adopt integrated control programs, that combine field sanitation, crop rotation, planting clean seed, cultural practices, limited use of chemicals, and a greater reliance upon resistant cultivars.

1.5.2 Common Blight of Beans

1.5.2.1 The Pathogen

Common bacteria blight is caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. The pathogen is also referred as *Xanthomonas phaseoli* (Allen, 1987). It has two variants: *X. phaseoli* (Erw. Smith) Dowson and a brown pigment producing, *X. phaseoli* var.

fuscans (Burk.) Starr et Burk (Saettler, 1989). Now the common blight bacterium is referred as *Xanthomonas axonopodis* pv. *phaseoli* (Vauterin *et al.*, 1995) (Xap). The bacterium Xap is a gram negative straight rod, strictly aerobic, bacterium that is motile by a polar flagellum and produces a yellow, water-insoluble carotenoid and mucoid growth on nutrient glucose agar (Saettler, 1989). The synonyms to Xap are *Bacillus phaseoli* E.F. Sm., 1897; *Pseudomonas phaseoli* E.F, Sm., 1901; *Bacterium phaseoli* E.F Sm, 1905; *Phaytomonas phaseoli* (E.F.Sm.) Berger *et al.*, 1923 and *Phaytomonas phaseoli* var. *fuscans* Burk, 1930. (Walker, 1950).

1.5.2.2 Host Range

The pathogen Xap is known to infect common bean (*Phaseolus vulgaris* L.), scarlet runner bean (*P. coccineus* L.), urd bean (*Vigna mungo* (L.) Hepper), mung bean (*V. radiata* (L) Wilezek var. *radiata*), tepary bean (*P. acutifolius* A. Gray var. *acutifolius*), *V. aconitifolia* (Jacq.) Marechal, V. *angularis* (Willd.) Ohwi et Ohasi, *Lablab purpureus* (L.) Sweet, *Strophostyles helvola* (L.) Elliott, soybean (*Glycine max* (L) Merrill), *Mucuna deeringiana* (Bort.) Merrill, *Lupinus polyphyllus* Lindl, and cowpea (*V. unguiculata* (L.) Walp. ssp. *unguiculata*) (Vakili *et al.*, 1975; Zaumeyer and Thomas, 1957, cited in Saettler, 1989).

1.5.2.3 Geographic Distribution and Economic Importance

Common blight was first described in the United States by Beach in New York and by Halsted in New Jersey in 1892 (Walker, 1950). The disease also occurred in Canada (Walker, 1950). Saettler recorded that common blight disease is now distributed worldwide.

The bacteria can cause a yield loss of more than 40% (Allen, 1987). The two variants of the bacteria were reported to damage 75% of Michigan's 265 000 hectares of navy beans, with 10-20% yield reductions and in Ontario, Canada a yield loss of 38% was reported (Saettler, 1989).

1.5.2.4 Disease Cycle and Epidemiology

The common blight pathogen over-winters in the seed. When infected seed is planted, the pathogen develops in the seed coat of the germinated seed and contaminate the surface of the expanded cotyledon. It penetrates through rifts in the cuticle and progress intercellularly until the vascular system is reached (Hungerford *et al.*, 1929 cited in Walker, 1950).

The bacterium also over-winters in infected plant debris (Burkholder, 1930, cited in Saettler, 1989). Primary infection may occur through stomata by inoculum from infected debris (Walker, 1950) or from volunteer plants present in field (Saettler, 1989). Saettler (1989) pointed out that infected bean residue is not always an important primary inoculum source of the pathogen; but in tropical bean-growing regions, infected residue may be important because of the opportunities for bacteria to multiply and survive as epiphytes on perennial hosts, and because of the practice of intercropping.

Secondary inoculum is spread from primary lesions by irrigation water (Steadman *et al.*, 1975, cited in Saettler, 1989), wind-born rain, dust, implements, man and animals (Walker, 1950).

The pathogen causes great damage to plants at 28^0C (Goss, 1940; Patel and Walker, 1963; Mack and Waller, 1974, cited in Saettler, 1989). Saettler (1989) stated that the temperature range for optimum *in vitro* growth to be 28-32^0C. Growth stops at temperature as low as 16^0C. High humidity also favours the disease (Sutton and Wallen, 1970, cited in Saettler, 1989).

1.5.2.5 Symptoms

Symptoms on leaves first appear as water soaked spots, which enlarge and frequently coalesce with adjacent lesions (Saettler, 1989). A narrow zone of yellow tissue surrounds the blight areas and a black exudate is associated with the lesions on lower leaf surface.

The pathogen from infected leaves reaches vascular elements and colonizes xylem tissue, which may cause plant wilting by plugging vessels or disintegrating cell walls (Saettler, 1989). Plants that grew from infected seed may develop stem girdling or joint rot, which may cause the plant to break at the node (Zaumeyer and Thomas, 1957 cited in Saettler, 1989).

Lesions also appear on pods. The lesions can be small, greasy looking spots, in others water soaked, brown to reddish in colour and may often coalesce (Walker, 1950; Gubler, 1990). Yellow bacterial exudate may be present on pod lesions in humid weather (Dillard and Legard, 1991).

Different symptoms are manifested on seed infected by Xap. Infected white-coloured seed show butter-yellow spots (Zaumeyer and Thomas, 1957; Saettler and Perry, 1972, cited in Saettler, 1989; Davis *et. al.*, 2002). Severely infected seeds may give rise to stunted seedlings, with damaged growing tips, which finally dies (Zaumeyer and Thomas, 1957, cited in Saettler, 1989).

1.5.2.6 Seed Infection and Phytosanitary Significance

The pathogen infects the seed coat and may pass into the cotyledons which are subsequently invaded when the seed germinates (Zaumeyer and Thomas, 1957, cited in Neergaard,1977). Both external contamination and internal infection of seeds result in infected plants (Saettler and Perry, 1972).

The bacterium Xap is seed borne and is introduced into a new region via a contaminated seed. Low levels of seed infection with the bacterium can result in heavy field infections and might cause severe crop losses under favourable environmental conditions (Zaumeyer and Thomas, 1957, cited in Neergaard,1977).

1.5.2.7 Control

Planting certified, disease-free seeds can control common bacterial blight of bean (Dillard

and Legard, 1991). Clean seed must be produced in regions free of pathogen or where environmental conditions discourage disease development (Saettler, 1989).

Infested bean residues are better for survival of the pathogen when they are on the soil surface than when they are buried (Dillard and Legard, 1991). Therefore, incorporation of plant residue into the soil can be a means of control of the disease.

Crop rotation also helps in control of the pathogen. Rotation of susceptible crops with resistant ones gives time for the Xap population in bean debris with in a field to decline (Saettler, 1989). A minimum of two-year rotation out of the bean would allow the crop residue to completely decompose (Dillard and Legard, 1991).

Several chemical treatments are used to control common blight of bean, though not a complete control. Surface contamination of seed coat can be reduced by streptomycin treatment (Dillard and Legard, 1991). When streptomycin was applied in laboratory and field tests, only marginal control was observed; it translocated within the plant but not into the developing seed (Mitchell *et al.*, 1952; 1953; and 1954 cited in (Saettler, 1989). On bean foliage, copper-based bactericides will reduce epiphytic population of bacterial pathogens and also disease severity when applied as a preventative treatment (Dillard and Legard, 1991).

1.5.3 Bean Common Mosaic Virus (BCMV)

1.5.3.1 The Pathogen

The virus was originally named as 'bean mosaic virus' before the name was changed to 'bean common mosaic virus' (BCMV) to differentiate it from bean yellow mosaic virus (Pierce, 1934 cited in Morales, 1998). Bean Common Mosaic Virus consists of filamentous particles about 750 nm long and 14 nm wide containing single stranded RNA (Morales, 1998). The virus induces cytoplasmic inclusions that appear as cylindrical pinwheels in the light or electron microscope (de Camargo *et al.*, 1968; Hoch and Provvidenti, 1978; Valdes *et al.*, 1982, cited in Galvez and Morales, 1989). The BCMV

particles are strongly immunogenic and there is a report of antisera with titres of 1/2000 in precipitin (Morales and Boss, 1988, cited in Dharma, 1994). BCMV is closely related serologically to AzMV, BlCMV, PStV, three potyvirus isolates from soybean in Taiwan (Taiwo and Gonsalves, 1982; Green *et al.*, 1986; Tsuuchzaki and Omura, 1987; Mink and Silbernagel, 1992; Vetten *et al.*, 1992a,b, cited in Dharma, 1994).

1.5.3.2 Host Range

Bean Common Mosaic Virus reported hosts include common bean (*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.), tepary bean (*P. acutifolius* var. *acutifolius*), *Vigna angularis* (Willd.) Ohwi et Ohasi, *V. aconitifolia* (Jacq.) Marechal, *V. umbellata* (Thumb.) Ohwi et Ohashi, urd bean (*V. mungo* (L.) Hepper), scarlet runner bean (*P. coccineus* L.), siratro (*Macropitilium atropurpureum* (D.C) Urb.), *V. radiata* (L.) Wilczek var. *radiata*, *P. polyanthus* Greenman, *Vigna anguiculata* spp., *Unguiculata* var. *sesquipedalis* (L.) Verdc., cowpea (*V. unguiculata* (L.) Walp. spp. *unguiculata*), broad bean (*Vicia faba* L.), *Crotalaria spectabilis* Roth., *Canavalia ensiformis* (L.) D.C., *Lupinus ulbus* L., *Nicotiana clevelandii*, *Macropitilium lathyroides* (L.) Urb., pea (*Pisum sativum* L..), alfalfa (*Medicago sativa* L.), *Lablab purpureus* (L.) Sweet, common clover (*Trifolium pratense* L.), and *Rhynchosia minima* (L.) DC. (Zaumeyer and Thomas, 1957; Bos, 1971; Kaiser *et al.*, 1971; Ordosgoitty, 1972; Kaiser and Mossahebi, 1974; Meiners *et al.*, 1978, cited in Galvez and Morales, 1989).

1.5.3.3 Geographic Distribution and Economic Importance

As Galvez and Morales (1989) described it, BCMV was one of the first virus diseases reported in the world when Iwanoski in 1894 observed it in the Soviet Union. It was first isolated from infected bean (*Phaseolus vulgaris*) in New York, USA, by Stewart and Reddick (1917) (Dharma *et al.*, 1994). The virus is now economically important throughout Africa, Europe, North America and Latin America (Galvez and Morales, 1989).

Plant infection may reach 100% in the field and yield losses range from 35-98% (Galvez

and Morales, 1989).

1.5.3.4 Disease Development and Epidemiology

Bean Common Mosaic Virus is seed-borne and new plants produced from infected seeds may develop the disease. Seed transmission is primary source of infection and it may vary from 30–50% and in *P. vulgaris* it may reach up to 83% (Smith, 1972; Brunt, 1990). BCMV particles can survive in bean seed for at least 30 years (Zaumeyer and Thomas, 1957, cited in Galvez and Morales, 1989). BCMV can be transmitted mechanically. Leaves infected by the virus can be used as inoculum by homogenizing in water or buffers such as potassium phosphate, and are applied to leaves of healthy plants (Morales, 1979, cited in Galvez and Morales, 1989). The disease particles can also be transmitted in pollen grains, ovules, and flowers of infected plants (Zaumeyer and Thomas, 1957; Wilson and Dean, 1964; Ekpo and Saettler, 1974; cited in Morales, 1998).

Insect vectors are responsible for secondary transmission of the virus. Several aphid species, such as *Myzus persicae* and *Aphis fabae* transmit the virus in a "nonpersistent" manner (Morales, 1989).

1.5.3.5 Symptoms

Bean Common Mosaic Virus induces several symptoms, which vary depending on the host cultivar, environmental conditions and virus strain (Dharma *et al.*, 1994).

Mosaic symptoms of well-defined dark and light green areas, often in vein-banding are characteristic foliar symptoms induced by BCMV (Morales, 1989). Other symptoms include downward cupping along the main vein of each leaflet, green vein-banding, blistering and malformation of leaves, reduced plant size, mottled and malformed pods (Zitter and Provvidenti, 1984).

Some strains of BCMV challenge the resistance to mosaic-inducing strains of BCMV and produce "black root" symptoms in the plant (Morales, 1989). These symptoms initially

appear as a progressive vein necrosis of the young trifoliolate, which then die. Latter wilting of the older leaves followed by death of the entire plant occurs (Galvez and Morales, 1989).

Local lesions are observed at temperature range of 16-28^{0}C (Morales, 1989). These lesions, which manifest as reddish to dark brown necrotic ring-shaped lesions or spots, may be induced by mechanical inoculation or aphid transmission (Galvez and Morales, 1989).

Some bean genotypes, which are infected by BCMV, may remain symptomless (Morales, 1989). Tolerant cultivars may show only slight leaf narrowing or rolling (Brunt *et al.*, 1990).

1.5.3.6 Seed Infection and Phytosanitary Significance

Bean common mosaic virus is one of the economically important bean diseases that are seed-borne. The virus is mostly located in the embryo (Dharma *et al.*, 1994). The seed coat also carries the virus (Neergaard, 1977).

Bean common mosaic virus has high incidence of seed transmission which is thought to be the most important factor for initial crop infection and its world wide distribution (Dharma et *al.* 1994).

1.5.3.7 Control

Controlling the vector population is crucial since vector aphids transmit BCMV. There is correlation between planting date and virus incidence associated with aphid population levels (Bruke, 1964, cited in Galvez and Morales, 1989).

It may be necessary to control aphids with insecticide in order to reduce transmission of BCMV from other infected bean plants or weed hosts (Sarchez and Pinchinot, 1974, cited in Galvez and Morales, 1989). However, in a clean seed production operation, chemical control of aphids is not recommended since acquisition and transmission of BCMV by

aphids takes place within less than 30 seconds (Morales, 1989). In some cases, treatment with chemicals increased virus infection due to increased excitation of sprayed aphids (Budnik, 1995).

Due to the difficulty of the vector control, the incorporation of genetic resistance to BCMV is the most recommended and widely practiced control method (Morales, 1989). Resistance was developed after the discovery of the cultivar Robust (Galvez and Morales, 1989). Bean cultivars possessing the dominant I gene have hypersensitive resistance to systemic mosaic and develop systemic necrosis (black root) and/or local lesions when plants are inoculated with one or more of the necrosis inducing strains of BCMV (Galvez and Morales, 1989; Morales, 1998). Morales (1998) stated that breeding for common mosaic resistance in *P. vulgaris* has been the main objective of most important bean breeding programs around the world.

The virus is primarily spread and introduced to a new area through an infected seed. Planting certified disease-free seed is an effective control measure of BCMV as the virus can survive in seed for more than 30 years and can resist heat treatments hot enough to kill the seed (Mercure, 1998).

1.5.4 The Bean Weevil

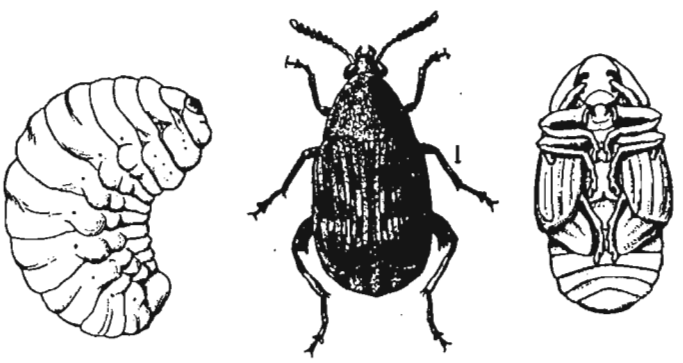
Bean weevil (*Acanthoscelides obtectus* (Say)) is a destructive storage weevil. Bean weevil, together with the Mexican Dry Bean weevil (*Zabrotes subfasciatus* (Boheman)), are the two major post harvest pests of dry beans. Bean weevil, originally a native of America, is found all over the world today (Skaife, 1918; Hoffmann *et al.*, 1962, cited in Multon, 1988).

1.5.4.1 The insect

The bean weevil belongs to the phylum Arthropoda, class Insecta, order Coleoptera and family Bruchidae (Hall, 1970; Anonymous, 1994). The bruchids, though they are referred as weevils, they do not have the characteristic snout of the weevil. The bean weevil is a

tiny insect, is about 0.4cm long. It is brownish-grey, and has a short broad snout and shortened elytra (wing covers) with ashy-grey spots (Fig. 1).

Skaife (1918) reported that the female deposits her eggs inside ripe, dry bean pods. To do this the female first gnaws a slit about one millimetre long in the ventral suture of the pod and inserts her ovipositor inside the slit to drop her eggs loosely inside the pod. He also stated that the female ignored the green pods and oviposited in the dry ones as the succulent, green pods are too thick to allow the insect to gnaw though them in order to reach the interior.



a b c

Figure 1. The different stages of the bean weevil. a) larva b) adult beetle and c) pupa. (Picture taken from Skaife, 1918)

1.5.4.2 The eggs

The egg is oval in shape, translucent white in colour, and measures 0.8mm by 0.3mm. According to Skaife (1918) under laboratory conditions a female could deposit up to 43 eggs. In his study, eggs hatched in from 10-13 days in summer and from 20-24 days during winter. Multon (1988) reported that a female could deposit 40-70 eggs under

optimum conditions, that is at a temperature of 27^0C and a relative humidity of 90%.

1.5.4.3 The larva

The larva has long slender legs and bears a number of long stiff hairs. It can move about actively by means of its legs and bristles (Skaife, 1918) (Fig.2).

The larva burrows into the seed or takes advantage of any crack in the integument of the bean and crawls beneath it in order to enter (Skaife, 1918). It takes 24 hours for the larva to bore into a dry bean seed and boring occurs at particular sites where a bean touches some other surface (Quentin *et al.*, 1991). The larvae of the last stage cut a circular flap in the epidermis leaving only the cuticle, which forms a very thin covering called the operculum (Multon, 1988). Skaife (1918) reported that the larva reached maturity in 3-4 weeks during summer and in 6-8 weeks during winter. The pupal stage lasted eight to eleven days in summer and 18-25 days in winter.

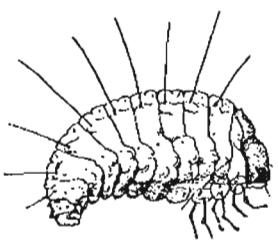


Figure 2. Post embryonic larva of the bean weevil. (Picture taken from Skaife, 1918)

1.5.4.5 The beetle

The adults (beetles) come out of their cells after a few days in summer, while in winter, they remain motionless in their cells (Skaife, 1918). Adult beetles are short lived and do not feed on stored produce (Hall, 1970). Hall stated that the life cycle is completed in about four weeks at 30^0C and 70% RH. Longevity of the adult beetle is much longer in the field or with artificial liquid feeding (Zaazou, 1948, cited in Multon, 1988).

1.5.4.6 Host plants

The bean weevil can develop on a range of seeds from cowpea (*Vigna unguiculata* L.), broad bean (*Vicia faba* L.), kidney bean (*Phaseolus vulgaris* L.), chick pea (*Cicer arietinum* L.) and wild pea (*Vicia Americana* Muhl. Ex Willd.) (Krischik and Burkholder, 1997).

1.5.4.7 Control

Low temperature (less than 10^0C) storage eliminates the insect in any of its forms, as it is only adapted to higher temperatures of 20-32^0C (Jones, 2002). Karapetyan (1983) worked on the mortality of the bean weevil at all stages in relation to cold temperature. He found that 100% mortality of all stages of the weevil was possible when beans were stored at -11^0C for at least one day, -9^0C for at least 3 days, -8^0C for at least 4 days, -6^0C for at least 12 days or -1^0C for at least 15 days.

Ishimoto (2000) reported that high levels of resistance against the storage pests of common bean have been found in wild common beans collected in Mexico. Resistance, inherited by dominant gene, has recently become available in commercial bean types (Jones, 2002).

Beans mixed with lime or dry pulverized soil can be protected from bean weevils. The fine dust-particles suffocate them by clinging to the hairy covering of their bodies (Skaife, 1918). He also stated soaking seeds in paraffin and allowing them to dry would kill the grubs already present in the beans and protect the beans against future infestation. Edible vegetable oils are also an effective control, which act by penetrating the eggs until they are destroyed and reducing oviposition (Jones, 2002).

Chemical control can be also applied to control the pest. Spraying pods with insecticides in the field (Kr''steva, 1982) and fumigation of stored bean seeds with hydrogen phosphide (Bogs, 1981) is recommended for control of the pest.

Skaife (1918) mentioned a natural enemy, *Pediculoides (Heteropus) ventricosus* Newport, which attacks and kills a small proportion of the larvae and pupae. Gafurova and Kurbatskaya (1980) observed total mortality of the bean weevil feeding on food treated with endotoxin and spore suspension of *Aspergillus ochraceus*.

REFERENCES

Anonymous. 1994. Bean weevil in: The Columbia Encyclopedia, 5th ed. Columbia University Press.

Allen, D.J. 1987. Principal diseases in Africa. CIAT, Cali, Colombia.

Aujla, S.S., Grewal, A.S. and Sharma, I. 1983. Yield and vigour components of wheat as affected by Karnal bunt pathogen (*Neovossia indica*). *Journal of Punjab Agricultural Research University* 20:231-235.

Bansal, R., Singh, D.V., and Joshi, L.M. 1984. Effect of Karnal-bunt pathogen (*Neovossia indica* (Mitra) Mundkur) on weight and viability of wheat seed. *Indian Journal of Agricultural Science* 54: 663-666.

Bogs, D. 1981. The control of the bean weevil (*Acanthoscelides obtectus* Say) with hydrogen phosphide. *Nachrichtenblatt fur den Pflanzenschutz in der DDR,* Vol.35: 210.

Brunt, A., Crabtree, K., and Gibbs, A. 1990. Viruses of Tropical Plants. CAB International, Wallingford, UK.

Budnik, K. 1995.The epidemiology and control of capsicum viruses in Natal. University of Natal, Pietermaritzburg.

Burris, J.S., A.H. Wahab, and O.T. Edje. 1971. Effects of seed size on seedling performance in soybeans. I. Seedling growth and respiration in the dark. *Crop Science* 11: 429-436.

Campbell, R. 1985. Plant Microbiology. Edward Arnold, London.

CBS, 2001. South African seed certification scheme: dry bean producers. Available: http://www.beans.co.za/a-fr4c.htm

Copeland, L.O. and McDonald, M.B. 1995. Principles of Seed Science and Technology, 3rd ed. Chapman and Hall, New York.

Davis, R.M., Hall, A.E., and Gilbertson, R.L. 2002. UC Pest Management Guidelines. Dry Beans. Halo Blight. Available: http://www.ipm.ucdavis.edu/PMG/r52101211.html

Delouche, J.C. and Baskin, C.C. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Science and Technology* 1: 427-452.

Dennis, R.W.G. 1952. Plant diseases in orchard, nursury and garden crops. McDonald, London.

Dharma, D.S., Ward, C.W. and Brunt, A.A. 1994. The Potyviridae. CABI, UK.

Dillard, H.R. and Legard D.E. 1991. Vegetable MD Online. Vegetable Crops. Bacterial Diseases of Beans. Available:

http://vegetablemdonline.ppath.cornell.edu/factsheets/Beans_Bacterial.htm

Gafurova, V.L., and Kurbatskaya, Z.A. 1980. The influence of endotoxins of entomopathogenic fungi on their virulence. *Mikrobiologicheskii Zhurnal* 5:591-595.

Galvez, G.E. and Morales, F.J. 1989. Aphid-transmitted viruses. In H.F. Schwartz, and M.A. Paster-Corales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Grogan, R.G. and Kimble, K.A. 1967. The role of seed contamination in the transmission of *Pseudomonas phaseolicola* in *Phaseolus vulgaris*. *Phytopathology* 57: 28-31.

Gubler, W.D., McCain, A.H. Ohr, H.D., Paulus, A.O. and Teriotdale, B. 1990. California Plant Disease Handbook and Study Guide for Agricultural Pest Control Advisors, 4th ed. UC, Division of Agriculture and Natural Resources U.S.A.

Guthrie, J.W. 1970. The detection and identification of *Pseudomonas syringae* pv. *phaseolicola* in *Phaseolus vulgaris*. *Proceedings of International Seed Testing Association* 35: 89-93.

Guthrie, J.W. Huber, D.M., and Fenwick, H.S. 1965. Serological detection of halo blight. *Plant Disease Reporter* 49: 297-299.

Hall, D.W. 1970. Handling and storage of food grains in tropical and subtropical areas. FAO, Rome, 92-93.

Hall, R. 1991. Bean diseases. In R. Hall, (ed), Compendium of bean diseases. APS Press, U.S.A.

Harveson, B. (undated). Halo blight of dry bean. Available: http://pdc.un.l.edu/drybean/Haloblight/print%20

Hibbard R.P., and Miller, E.V. 1928. Biochemical studies on seed viability. I. Measurement of conductance and reduction. *Plant Physiology* 3: 335-352.

Hoffmann, A., Labeyrie, V., Balachowsky, A.S. 1962. Famille des Bruchidae. In: Balachowsky, A.S. (ed), Traite d'entomologie appliquee a l'agriculture. Masson, Paris, Vol. 1, 434-494.

Ishimoto, M. 2000. Evaluation and use of wild *Phaseolus vulgaris* species in breeding. The Seventh Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan, International Workshop on Genetic Resources, Ibaraki, Japan, 13-15 October, 1999: Part 1, wild legumes. pp. 183-190.

ISTA. 1995. Handbook of Vigour Test Methods (ed. J. Hampton and D. Tekrony), 3rd edition. International Seed Testing Association, Zurich, Switzerland.

ISTA. 1999. International rules for seed testing. Supplement to Seed Science and

Technology 27: 155-1999.

Jones, A.L. 2002. Phaseolus bean: post-harvest operations. Available at: http://www.fao.org/inpho/compend/text/ch04-03.htm

Karapetyan, A.P. 1983. The ecology and harmfulness of the bean weevil *Acanthoscelides obtectus* (Say) in the conditions of the Armenian SSR. *Biologicheskii Zhurnal Armenii* 8: 647-651.

Krischik, V. and Burkholder, W. 1997. Stored-product insects and biological control agents. Available:

http://www.okstate.edu/OSU-Ag/agedcm4h/pearl/e912/ch13/ch13f23.htm

Kr''steva, L. 1982. The bean weevil and its control. *Rastitelna Zashchita* 10: 47-48.

McDonald, M.B., Gutormson, T. and Turnipseed, B. 2001. Eds. Seed Technologist Training Manual. Society of Commercial Seed Technologists,

Mercure, P.S. 1998. Bean viruses. Available: http://www.hort.uconn.edu/ipm/veg/htms/bnvirus.htm

Morales, F.J. 1989. Bean common mosaic. CIAT, Cali, Colombia.

Morales, F.J. 1998. Present status of controlling bean common mosaic virus. In A. Hadidi, R.K. Khetarpal, and H. Koganezawa, (eds), Plant Virus Disease Control. APS Press, Minnesota.

Multon, J.L. 1988. Preservation and storage of grains, seeds and their by-products: Cereals, oilseeds, pulses and animal feed. Lavoisier, USA. pp378-380.

Neergaard, P. 1977. Seed pathology, Vol. 1. Macmillan, England.

Pastor-Corrales, M.A. and Tu, J.C. 1989. Anthracnose. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics. CIAT, Cali, Colombia.

Perry, D.A. 1978. Report of the vigour test committee 1974-1977. *Seed Science and Technology* 6: 159-181.

Powell, A.A. 1988. Seed vigour and field establishment. *Advances in research and technology of seeds* 11:29-61.

Quentin, M.E., Spencer, J.L. and Miller, J.R. 1991. Bean tumbling as a control measure for the common bean weevil, *Acanthoscelides obtectus*. *Entomologia Experimentalis et Applicata* 60: 105-109.

Rai, R.C. and Singh, A. 1978. A note on the viability of wheat seeds infected with Karnal bunt. *Seed Research* 6: 188-190.

Saettler, A.W. 1989. Common bacterial blight. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Saettler, A.W. and Perry, S.K.1972. Seed transmitted bacterial diseases in Michigan navy (pea) beans, *Phaseolus vulgaris*. Plant Disease Reporter 56:378-381.

Schwartz, H.F. 1989. Halo blight. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Singh, D. 1980. A note on the effect of Karnal bunt infection on the vigour of wheat seed. *Seed Research* 8: 81-82.

Singh, P.S. 1999. Development in plant breeding Vol.7. Common bean. Improvement in the Twenty-First Century. Kluwer Academic Publishers, Netherlands.

Singh, R.A., and Krishna, A. 1982. Susceptible stage for inoculation and effect of karnal

bunt on viability of wheat seed. *Indian Phytopathology* 35: 54-56.

Skaife, S.H. 1918. Pea and bean weevils. *Bulletin of the Union of South Africa, Department of Agriculture* 12: 13-20.

Smith, K.M. 1972. A textbook of plant virus diseases, 3rd ed. Academic Press, New York.

Spence, N.J. and Walkey, D.G.A. 1994. Bean common mosaic virus and related viruses in Africa. *NRI Bulletin* 63, Chatham, UK.

Taylor, J.D. and Dudley, C.L. 1977. Seed treatment for control of halo blight beans (*Pseudomonas phaseolicola*). *Annals of Applied Biology* 85:223-232.

Taylor, J.D., Dudley, C.L. and Presly, L. 1979. Studies of halo blight infection and disease transmission in dwarf beans. *Annals of Applied Biology* 93: 267-277.

Vauterin, L., Hoste, B., Kersters, K. and Swings, J. 1995. Reclassification of Xanthomonas. International Journal of Systematic Bacteriology 45:472-489.

Vennette, J.R. 1985. Reduction of bean seed emergence by vacuum infiltration with bacterial pathogens. *Phytopathology* 75: 1385.

Walker, J.C. 1950. Plant pathology. McGraw-Hill Book Company, U.S.A.

Walker, J.C. and Patel, P.N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. *Phytopathology* 54: 140-141.

Warham, E.J. 1990. Effect of *Tilletia indica* infection on viability, germination, and vigour of wheat seed. *Plant Disease* 74: 130-132.

Webster, D.M., Atkin, J.D. and Cross, J.E. 1983. Bacterial blights of snap beans and their control. *Plant Disease* 67: 935-940.

Zaazou, H. 1948. Oviposition of the bean weevil. *Bulletin of the Society Fouad Ier of Entomology* 32: 343-361.

Zitter, T.A. and Provvidenti, R. 1984. Vegetable Crops. Virus diseases of snap and dry beans. Vegetable Crops. Fact Sheet, pp 729-730.

CHAPTER TWO

USE OF SELECTIVE MEDIA TO SCREEN BEAN SEED FOR *Xanthomonas axonopodis* pv. *phaseoli* and *Pseudomonas syringae* pv. *phaseolicola*

2.1 INTRODUCTION

Common and halo blight of beans are caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (Xap), *and Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young *et al.* (Psp) respectively (Saettler, 1989; Schwartz,1989). Both are major seed-borne diseases of beans (*Phaseolus vulgaris* L.) worldwide (Saettler, 1989; Schwartz, 1989). Xap and Psp are serious pathogens of beans because low levels of primary infection, one infected plant per hectare, can cause an epidemic when conditions are favourable (Webster *et al.*, 1983). For a susceptible navy bean cultivar, it was found that at least 10^3 viable bacteria per seed were necessary for seedling infection (Weller and Saettler, 1980). Experiments also show that a level of one Psp infected plant in 10,000-16,000 was the limit above which pod lesions in the vegetable crop could reach an unacceptable level for susceptible cultivars grown in conditions favourable for the disease (Taylor *et al.*, 1979).

Common and halo blights of beans cause economic losses due to seed yield reduction and loss of seed marketability (Saettler, 1989; Schwartz, 1989). Goodwin (1992) found that photosynthetic rate of Xap diseased leaves had dropped to approximately one half of healthy leaves when only 15 to 20% of the leaf area was covered by lesions. Magyarosy and Buchanan (1975) also showed that bean plants infected with Psp showed reduced photosynthetic C assimilation within two hours of infection.

There are several studies on the survival of the blight pathogens in the soil and soil debris. Psp and Xap were found to survive for 10 months both in buried and unburied bean straw in soil (Schuster, 1970, cited in Wimalajeewa and Nancarrow, 1980). Whereas experiments by Wimalajeewa and Nancarrow (1980) show that the blight pathogens do not over-winter in the soil, either freely or in association with infested debris.

The bacteria Xap and Psp can grow epiphytically on tolerant bean genotypes without causing symptoms (Cafati and Saettler, 1980; Stadt and Saettler, 1981). Weller and Saettler (1980) also recorded that Xap can be present as an epiphyte on bean pods, seeds and leaves.

The bacterial common blight may also over-winter on weeds. Cafati and Saettler (1980) suggested that weeds, as well as associated crops such as maize, could function as important inoculum sources of common blight in tropical and semi-tropical bean production regions.

The most important means of survival for Xap and Psp is seed-borne contamination (Saettler, 1989; Schwartz, 1989). Contamination can be both internal and external (Grogan and Kimble, 1967; Guthrie, 1970; Taylor *et al.*, 1979; Saettler, 1989). Studies show that a few infected seeds are sufficient to initiate a general epidemic under favourable conditions (Walker and Patel, 1964; Sutton and Wallen, 1970 cited in Audy *et al.*, 1996). As a result of the tremendous disease potential in these seed-borne bacteria, the use of pathogen free seeds becomes the only practical control for these serious bacterial blight diseases.

Seed certification programs use several methods to detect contaminated seed lots and to isolate the pathogen from field samples.

Many countries and states have established strict quarantine regulations for bean blights, which involve field inspection and laboratory analysis (Webster *et al.*, 1983; Prosen *et al.*, 1993). Some of the laws enforced a zero tolerance for bacterial diseases found; any crop in which blight was found at the growing stage was destroyed. However, field inspections have had limited success and are considered to be less reliable (Mohan and Schaad, 1987).

Several other seed-testing procedures have been practiced, including plating seed soak liquid onto Medium B of King *et al.* (KB), inoculating bean plants with soak liquid from

cull seeds (Webster *et al.*, 1983), "Dome" test (Vennette, 1983, cited in Mohan and Schaad, 1987), direct serological assays (Guthrie *et al.*, 1965; Van Vuurde *et al.*, 1983; Trigalet and Bidaud, 1987 cited in Mohan and Schaad, 1987). Searching for relatively few pathogen cells of Psp in the presence of large number of saprophytes on KB medium does not work well (Mohan and Schaad, 1987). The test on culls is not foolproof, and some test results give false negatives, especially when disease incidence is low (Webster *et al.*, 1983). In the "Dome" test, where seeds soaked for 24 hours are vacuum infiltrated with the same solution and grown in a humid chamber for symptom development, high concentration of bacteria can produce lesions (Lahman and Schaad, 1985). Serological assays are not yet sufficiently specific and sensitive (Sheppard *et al.*, 1989; van Vuurde and Bovenkamp, 1989 cited in Audy *et al.*, 1996) and are not able to provide information on viability and pathogenicity of seed-borne inoculum (Schaad, 1982).

Several tests based on nucleic acid or DNA of the bacterial pathogens have been developed for their specificity, sensitivity and quick test duration. DNA hybridisation probes are highly specific tools for detection and identification of Xap and Psp (Gilbertson *et al.*, 1989; Schaad *et al.*, 1989). These probes were found to be effective with large numbers of target bacterial cells, but not for low pathogen populations in the presence of high populations of saprophytic bacteria (Prosen *et al.*, 1993). Polymerase chain reaction (PCR) – amplification assays developed for common (Audy *et al.*, 1994) and halo blights (Prosen *et al.*, 1993) are highly sensitive but the method requires time consuming steps for the extraction of bacterial DNA and the cost of Southern blots (Prosen *et al.*, 1993). Moreover, these methods do not differentiate between live and dead cells. BIO-PCR was developed to enable detection of live cells (Audy *et al.*, 1996), but is a time consuming procedure. To solve this problem, a rapid and sensitive PCR assay for simultaneous detection of seed-borne Xap and Psp was developed (Audy *et al.*, 1996). However, this method does not discriminate between live and dead pathogen cells.

The above nucleic acid based seed test methods, although they provide specificity, sensitivity and rapidity of testing, are expensive and require more sophisticated

laboratory equipment when compared to seed test based on selective or semi-selective media for detection of Xap and Psp. The DNA probes of PCR for Psp detection are based on the genes for phaseolotoxin production. However, a strain of *P.s.* pv. *syringae* was found to produce phaseolotoxin though the genotype is rare and may not be associated with bean plants (Tourte and Manceau, 1995). Therefore, selective and semi-selective media remain to be important for routine detection of bacteria including Xap and Psp.

In this study, two semi-selective media, Milk Tween (MT) and Modified Sucrose Peptone (MSP), for Xap/Psp and Psp respectively were compared for their plating efficiency to recover bacterial cells. A comparative test for *Xanthomonas axonopodis* pv. *phaseoli* to determine suitability of semi selective media was conducted in 2002, organized and prepared by ISTA/ISHI for several laboratories around the world. Results from this test conducted at Agricultural Research Council (ARC) in Roodeplaat, north of Pretoria, South Africa are presented here.

2.2 MATERIALS AND METHODS

2.2.1 Bacteria

Isolates of Xap and Psp were obtained from Mrs Tereza Goszczynska from the Agricultural Research Counsel (ARC) culture collection, at Roodeplaat (north of Pretoria). The bacteria in the culture collection were stored in ampoule by freeze dry method. Isolates used for plating were Sp75 for Psp and Sx70 for Xap.

To restore bacterial growth from their storage condition, a procedure described by the ARC was followed. The ampoules were wiped off with 70% ethanol and left for ten minutes to dry. The top of the ampoule was broken with a sterile metal rod. The cotton wool inside was removed aseptically and 0.5ml of sterile nutrient broth was added. The mixture was left for 20-30 min. Bacteria and broth were mixed using a pipette and 40µl of the suspension was plated on nutrient agar and milk Tween agar (MT) (Goszczynska and Serfontein, 1998) (Appendix 1, Part II). Plates were incubated at 27^0C for 2-4 days.

2.2.2 Plating assay of bacteria

Isolates of the two bacteria, Sx70 for Xap and Sp75 for Psp were plated on the two media MSP (Jansing, 1981) (Appendix 1, Part IV) and MT. Bacteria were grown on YDC (Appendix 1, Part I) medium for 48 hours at 28^0C. Bacterial growths were aseptically picked up with a sterile loop and added to 10ml of sterile broth in a bottle. A dilution series was prepared with the nutrient broth to get colonies of bacteria that can be counted (less than 300 colonies per plate). An amount of 100µl was plated on 9cm diameter petri dishes (in five replicates) for each media and was aseptically spread with a sterile glass rod. Plates were then incubated in an incubator at 28^0C for 3-4 days. Four experiments were done in the same way. Colonies grown in each plate were counted and results were computed statistically using Genstat General Analysis of Variance.

2.2.3 ISTA/ISHI Comparative Test

The materials, methods and procedures were specified by the ISTA/ISHI organizers of the comparative test and are shown in Part I of Appendix 1. In this test a new MT medium (MT_{new}) and XCP1 were used. For the sake of comparison, two media, the previous MT and Tween A (TSM) were also used. Composition of these media is shown in Part II and III of Appendix 1.

Twenty seed samples in plastic bags, each containing 1000 seeds with unknown infection levels, were soaked overnight in 20 flasks of 600ml sterile distilled water at 5^0C. A 10 fold dilution series (10^0-10^3) of each seed sample extract were prepared. An aliquot of 100µl was pippeted from each dilution and was plated onto two plates (replicates) for each of the four media (XCP1, MT, MT_{new} and TSM). Two known strains of Xap were plated as positive control. Plates were incubated at 28^0C for 3-5 days. After incubation, plates were evaluated according to colony characteristics. Colonies of Xap and other seed contaminants were counted and recorded. Results were analysed using Genstat General Analysis of Variance. Procedures and test information are described in Appendix 1 Part I.

2.3 RESULTS

2.3.1 Milk Tween (MT) and Modified Sucrose Peptone (MSP) Media

The difference in number of colonies for Psp in the two media MT and MSP was not significant (Table 1). However, the number of Xap colonies was significantly greater in MT than in MSP in the four experiments (Table 2).

2.3.2 *Xanthomonas campestris* pv. *phaseoli* Medium (XCP1), Milk Tween ($MT)_{new}$, Milk Tween (MT) and Tween A Medium (TSM)

At evaluation, there was no growth on media plated from five samples, plates from one sample were contaminated and plates from 14 samples had some growth. Out of the 14 samples, only plates from 7 samples had plenty growth. In this paper, the 7 samples were selected for making comparison of the four media for their ability to support growth of Xap. To make a comparison of growth of bean seed contaminants, plates from six samples were selected for their plenty growth. In each selected sample, comparison was done from colony counts in similar dilution levels, which had highest number of colonies that is less than 300.

The number of Xap colonies was significantly greater in TSM than the rest of the media, almost in all the samples analysed. XCP1, MT_{new} and MT were similar in number of the bacteria, except that in one the number of Xap was significantly greater in MT_{new} and XCP1 than in MT. Results are shown in Table 3. Analysis of the number of seed contaminants in the four media gave significantly greater number of contaminants in MT_{new} than in MT in three out of the six samples analysed. The media XCP1 had fewer contaminants than MT in two of the samples, while in the rest either it was greater or the difference was not significant. There were fewer contaminants in TSM than MT_{new} and XCP1 in four out of the six samples although the difference was significant only once. Results are shown in Table 4. Appendix 3 shows pictures of the bacteria Xap on the four media.

Table 1. Number of *Pseudomonas syringae* pv. *phaseolicola* colonies on the two media (MT and MSP)

No of experiment	No of colonies in media [1]			
	MT	MSP	LSD	cv%
1	144[a]	132[a]	13.15	6.5
2	109[b]	110[b]	22.03	15.6
3	12[c]	17[c]	5.65	27.3
4	14[d]	13[d]	6.00	30.0

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

Table 2. Number of *Xanthomonas axonopodis* pv. *phaseoli* colonies on the two media (MT and MSP)

No of experiment	No of colonies in media [1]			
	MT	MSP	LSD	cv%
1	151[a]	101[b]	13.02	7.1
2	78[c]	66[d]	9.58	10.4
3	17[e]	11[f]	5.00	27.6
4	19[g]	13[h]	5.02	24.9

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

Table 3. Number of *Xanthomonas axonopodis* pv. *phaseoli* colonies on the four media (XCP1, MT_{new}, MT and TSM)

Sample	No of Xap colonies in media[1]					
	XCP1	MT_{new}	MT	TSM	LSD	cv%
1	250^{a}	290^{a}	275^{a}	300^{a}	52.86	6.8
2	150^{b}	196^{b}	137^{b}	300^{c}	101.0	18.6
3	53^{d}	38^{d}	40^{d}	80^{e}	19.68	13.5
4	70^{f}	102^{fg}	97^{fg}	111^{g}	35.86	13.7
5	58^{hi}	37^{i}	29^{i}	80^{h}	36.61	26.0
6	96^{j}	109^{j}	114^{j}	181^{k}	32.92	9.5
7	66^{ln}	49^{l}	21^{m}	74^{n}	23.92	16.5

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

Table 4. Number of seed contaminant colonies on the four media (XCP1, MT_{new}, MT and TSM)

Sample	No of seed contaminant colonies in media[1]					
	XCP1	MT_{new}	MT	TSM	LSD	cv%
1	38^{a}	112^{b}	77^{c}	1^{d}	11.06	7.0
2	80^{e}	73^{ef}	50^{f}	50^{f}	28.1	15.1
3	9^{g}	54^{h}	50^{h}	75^{i}	8.44	6.5
4	4^{j}	4^{j}	14^{jk}	24^{k}	18.02	57.1
5	224^{l}	208^{l}	46^{m}	141^{m}	142.6	33.3
6	54^{n}	16^{o}	8^{p}	15^{o}	4.28	6.7

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

2.4 DISCUSSION

Selective and semi-selective media are useful to recover cells of desired organisms by inhibiting or suppressing the growth of undesired organisms (contaminants) from plant material or soil. While inhibiting growth of contaminants, the plating efficiency of the desired bacteria may also be reduced (Claflin *et al.*, 1987). When MT and MSP were compared for *Pseudomonas syringae* pv. *phaseolicola* (Psp) growth, they showed similar plating efficiency. This could be because of the same type and amount of antibiotics components in the two media. However, for *Xanthomonas axonopodis* pv. *phaseoli* (Xap), significantly higher plating efficiency was seen in MT than in MSP in the four experiments. This could be due to the preference of Xap for the special type of peptone in MT compared to the normal peptone in MSP. In seed testing laboratories, MT can therefore be used to detect both Psp and Xap from seed samples simultaneously.

According to the data from the ISTA comparative test study, the number of Xap colonies recovered in TSM was greater than any of the three media. In XCP1, MT_{new} and MT no significant differences were observed in all the six samples except one in which MT was found to be lower in recovery of the bacteria. In the case of contaminants, none of the media was found to be consistently lower in number, however, MT had lower number of contaminants than MT_{new} in five out of the six samples, and significantly different in three samples. From this it can be concluded that using MT would be better medium because it provided lower number of contaminants than MT_{new} while being similar in recovery of Xap colonies.

REFERENCES

Audy, P., Laroche, A., Saindon, G., Huang, H.C. and Gilbertson, R.L. 1994. Detection of bean common blight bacteria, *Xanthomonas campestris* pv. *phaseoli* and *X.c.* pv. *phaseoli* var. *fuscans*, using the polymerase chain reaction. *Phytopathology* 84: 1185-1192.

Audy, P., Braat, C.E., Saindon, G., Huang, H.C. and Laroche, A. 1996. A rapid and sensitive PCR-based assay for concurrent detection of bacteria causing common and halo blights in bean seed. *Phytopathology* 86: 361-366.

Cafati, C.R. and Saettler, A.W. 1980. Effect of host on multiplication and distribution of bean common blight bacteria. *Phytopathology* 70: 675-679.

Claflin, L.E., Vidaver, A.K. and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. *Phytopathology* 77: 730-734.

Gilbertson, R.L., Maxwell D.P., Hagedon D.J. and Leon, S.A. 1989. Development and application of a plasmid DNA probe for detection of bacteria causing common bacterial blight in bean. *Phytopathology* 79: 518-525.

Grogan, R.G. and Kimble, K.A. 1967. The role of seed contamination in the transmission of *Pseudomonas phaseolicola* in *Phaseolus vulgaris*. *Phytopathology* 57: 28-31.

Goszczynska, T. and J.J. Serfontein. 1998. Milk-Tween agar, a semi-selective medium for isolation and differentiation of *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas axonopodis* pv. *phaseoli*. *Journal of Microbiological Methods* 32: 65-72.

Goodwin, P.H. 1992. Effect of common bacterial blight on leaf photosynthesis of bean. *Canadian Journal of Plant Pathology* 14: 203-206.

Guthrie, J.W. 1970. The detection and identification of *Pseudomonas syringae* pv. *phaseolicola* in *Phaseolus vulgaris*. *Proceedings of International Seed Testing*

Association 35: 89-93.

Guthrie, J.W. Huber, D.M., and Fenwick, H.S. 1965. Serological detection of halo blight. *Plant Disease Reporter* 49: 297-299.

Jansing, H. 1981. Bean Halo Blight. In J. Jorgensen, (ed), ISTA Handbook for Seed Health Testing. Working Sheet No 66. State Seed Testing Station, Denmark.

Lahman, L.K. and Schaad, N.W. 1985. Evaluation of the "Dome Test" as a reliable assay for seed borne bacterial blight pathogens of beans. *Plant Disease* 69: 680-683.

Magyarosy, A.C. and Buchanan, B.B. 1975. Effect of bacterial infiltration on photosynthesis of bean leaves. *Phytopathology* 65: 777-780.

Mohan, S.K. and Schaad, N.W. 1987. An improved agar plating assay for detecting *Pseudomonas syringae* pv. *syringae* and *P.s.* pv. *phaseolicola* in contaminated bean seed. *Phytopathology* 77: 1390–1395.

Prosen, D., Hatziloukas, E., Schaad, N.W. and Panopoulos N.J. 1993. Specific detection of *Pseudomonas syringae* pv. *phaseolicola* DNA in bean seed by polymerase chain reaction-based amplification of phaseolotoxin gene region. *Phytopathology* 83: 965-970.

Saettler, A.W. 1989. Common bacterial blight. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Schaad, N.W. 1982. Detection of seed borne bacterial plant pathogens. *Plant Disease* 66: 885-890.

Schaad, N.W., Azad, H., Peet, R.C. and Panopoulos, N.J. 1989. Identification of *Pseudomonas syringae* pv. *phaseolicola* by a DNA hybridisation probe. *Phytopathology* 79: 903-907.

Schuster, M.L. 1970. Survival of bacterial pathogens of beans. Annual Report of the

Bean Improvement Co-operative, Vol.13: 68-70.

Schwartz, H.F. 1989. Halo blight. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

Sheppard, J.W., Roth, D.A. and Saettler, A.W. 1989. Detection of *Xanthomonas campestris* pv. *phaseoli* in bean. Pages 17-29 in: Detection of bacteria in seed and other plating material. A.W. Saettler, N.W. Schaad, and D.A. Roth, (eds). APS, St. Paul, MN.

Stadt, S.J., and Saettler, A.W. 1981. Effect of host genotype on multiplication of *Pseudomonas phaseolicola*. *Phytopathology* 71:1307-1310.

Sutton, M.D. and Wallen, V.R. 1970. Epidemiological and ecological relations of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* pv. *fuscans* on bean in south-western Ontario, 1961-1968. *Canadian Journal of Botany* 48: 1329-1334.

Taylor, J.D., Dudley, C.L. and Presly, L. 1979. Studies of halo blight infection and disease transmission in dwarf beans. *Annals of Applied Biology* 93: 267-277.

Tourte, C. and Manceau, C. 1995. A strain of *Pseudomonas syringae* which does not belong to pathovar *phaseolicola* produces phaseolotoxin. *European Journal of Plant Pathology* 101: 483-490.

Trigalet, A. and Bidaud, P. 1978. Some aspects of epidemiology of bean halo blight. Pages 895-902 in: *Proc. 4th International Conference of Plant Pathogenic Bacteria, Angers.*

Van Vuurde, J.W.L., Van den Bovenkamp, G.A. and Birnbaum, Y. 1983. Immunofluorescence microscopy and enzyme-linked immunosorbent assay as potential routine tests for the detection of *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas campestris* pv. *phaseoli* in bean seed. *Seed Science and Technology* 11:

547-559.

Vennette, J.R. 1983. Detecting seed-borne bacterial pathogens in bean seed: A semi-quantitative method. Vol. 7. Michigan Dry Bean Digest, Spring.

Walker, J.C. and Patel, P.N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. *Phytopathology* 54: 140-141.

Webster, D.M., Atkin, J.D. and Cross, J.E. 1983. Bacterial blights of snap beans and their control. *Plant Disease* 67: 935-940.

Weller, D.M. and Saettler, A.W. 1980. Evaluation of seed-borne *Xanthomonas phaseoli* and *X. phaseoli* pv. *fuscans* as primary inocula in bean blights. *Phytopathology* 70: 148-152.

Wimalajeewa, D.L.S. and Nancarrow, R.J. 1980. Survival in soil of bacteria causing common and halo blights of French bean in Victoria. *Australian Journal of Experimental Agriculture and Animal Husbandry* 20: 102-104.

CHAPTER THREE

SCREENING BEAN SAMPLES FOR BEAN COMMON MOSAIC VIRUS

3.1 INTRODUCTION

Bean common mosaic virus (BCMV) is an important disease worldwide. It is one of the earliest plant viruses described as pathogenic to common bean (*Phaseolus vulgaris* (L)) (Stewart and Reddick, 1917). It is known to be seed borne in common bean (*Phaseolus vulgaris* (L)), phasemy bean (*Macroptilium lathyroides* (L) Urb.), tepary bean (*P. acutifolius* Gray, var. *latifolius* Freeman), mung bean (*Vigna radiata* (L) Wilczek), and scarlet runner bean (*P. coccineus* L) (Reddick and Stewart 1919; Chamberlain, 1939; Kaiser and Mossahebi, 1974; Provvidenti and Cobb, 1975; Provvidenti and Braverman, 1976).

Bean common mosaic virus is a serious bean pathogen which may result in a high yield loss that might reach 98% depending on the virus strain, cultivar and time of infection (Galvez and Cardenas, 1974, cited in Schwartz and Pastor-Corrales, 1989). In the presence of high aphid population, 2%-6% seed infection was found to be able to initiate 100% plant infection. Planting virus-free seed most effectively controls bean common mosaic virus especially in areas with high vector incidence. The virus is prevented from spreading into a new area by testing imported or exported seed lots for presence of the virus. Seed tests include planting seeds for examination of symptom development and doing ELISA test.

In this study, seeds were harvested from plants artificially inoculated with two isolates of bean common mosaic virus in a greenhouse. Harvested seeds were planted in pots and seedlings were tested for the virus using symptom assessment and an ELISA test to see the suitability of the two testing schemes. Early and late matured seeds were also compared for their level (percentage) of seed transmission.

3.2 MATERIALS AND METHODS

3.2.1 Planting material

Bean seeds (*Phaseolus vulgaris*) of the variety PAN 146, certified to be free of *Pseudomonas syringae* pv. *syringae* (Pss), *Pseudomonas syringae* pv. *phaseolicola* (Psp) and *Xanthomonas axonopodis* pv. *phaseoli* (Xap) were used. The seed was harvested by Dr Rob Melis at Ukulinga Research Farm during the season 2002. These seeds were kept in plastic bags at a temperature of 20^0C for the duration of the experiment.

3.2.2 Plants in the greenhouse

Bean seeds were planted in pots with 20cm diameter and 17.5cm height in an insect free greenhouse. Three seeds were planted per pot and thinning was done 10 days later, leaving one plant per pot. Plants were grown at 25^0C and watered with an automatic sprinkler fertigation system with 3:1:3 (38) from Hortichem, Division of Ocean Agriculture. Plants were maintained under these disease free conditions for 12 days before inoculation.

3.2.3 Inoculum

Two strains of bean common mosaic (BCMV) were used as source of inoculum. The isolate 93/0083 was obtained from Ms Elize Jooste at Agricultural Research Counsel (ARC), Roodeplaat (north of Pretoria) in freeze dried form (here referred as BCMV-R). The second source was BCMV, here referred as BCMV-U, from infected seeds of common bean provided by Dr Rob Melis at Ukulinga Research Farm. The infected seeds were planted and leaves from plants with typical symptoms were collected for preparing the sap for inoculum (Fig. 1).

3.2.4 Inoculation

Thirty plants each were used for inoculation with the virus strains BCMV-R and BCMV-U. For BCMV-R the freeze dried material was diluted with phosphate buffer, pH 7.1 in 1:4 plant material to buffer ratio and about 0.25ml of the mixture was applied to a plant

by rubbing carborundum dusted primary leaves with a pestle. The same procedure was followed for inoculation of BCMV-U except that the ratio of diseased plant material (ground with mortar and pestle) to buffer was 4:1. Plants were inoculated twelve days after planting. Figure 2, 3 and 4 show virus symptoms in plants inoculated with the virus isolates respectively and Figure 5 shows positive ELISA test for inoculated plants.

3.2.5 Growing-on Test

Twelve seeds, in two replicates, each from the early matured and late matured (about two weeks later than the normal) seeds harvested from plants inoculated with the two virus isolates: BCMV-R and BCMV-U were planted in 24 pots of diameter 12.5cm and height 9cm, one seed per pot. Pots were placed in a shade house with sprinkler fertigation system as described above. Each plant was examined for development of symptom/symptoms after three weeks of planting. Plants which had at least one of the typical symptoms were considered positive.

3.2.6 Plant sample for ELISA Test

Twelve seedlings, in two replicates, each for the early and late matured seeds of the two virus isolates (BCMV-R and BCMV-U) were tested for the virus. A leaf sample of approximately 3g was taken from each seedling and placed in a microfuge tube. The hypocotyl (stem) was taken when seedlings did not emerge fully and develop into a normal plant. An extraction buffer of 500ml was added to each microfuge tube containing the plant sample. The leaf (or stem) sample was then ground in the microfuge tube using a sterile plastic rod to get the plant sap for ELISA test. Standard positive and negative controls were used. Positive control was diluted in 2ml of distilled water while negative control was diluted in 1ml according to the protocol of the manufacturers.

Figure 1. Plants grown from BCMV infected seeds from Ukulinga (BCMV-U)

Figure 2. Leaf mosaic and blistering symptoms on a BCMV-R inoculated plant

Figure 3. Vein banding and chlorosis symptoms on a leaf of a BCMV-U inoculated plant

Figure 4. Blistering symptom on a leaf of a BCMV-U inoculated plant

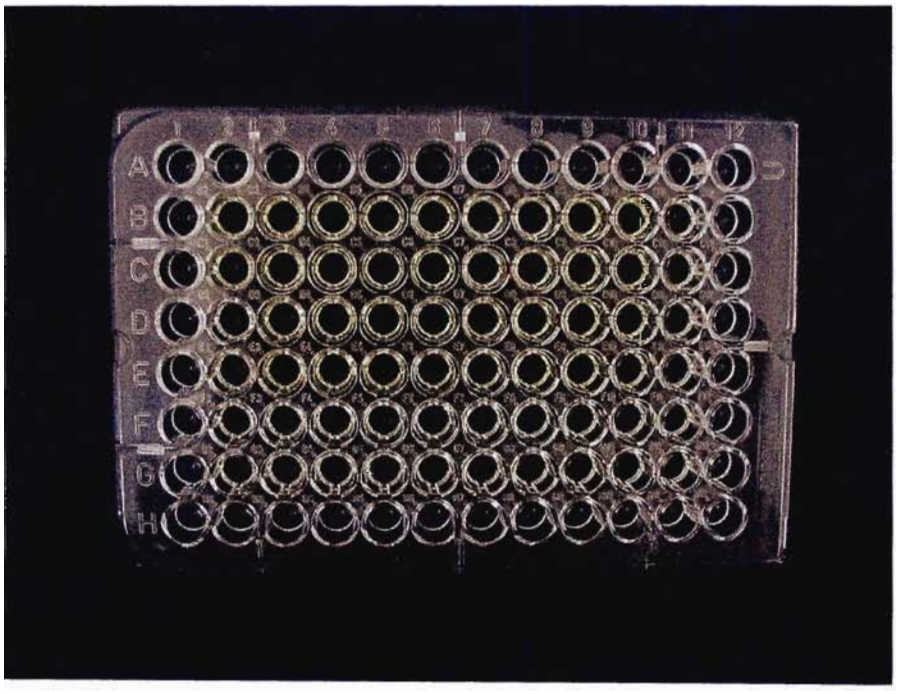


Figure 5. ELISA plate for ELISA test of BCMV-R and BCMV-U inoculated plants

3.2.7 ELISA Test

The reagents used in this study are from BIO-RAD PLANTEST ELISA*. Four ELISA plates were used for the 100 plant samples, each plate having the extraction buffer, standard positive and negative controls. The peripheral wells of the ELISA plates were not used. Plant sap of 100µl was pipetted into each well of an ELISA plate and plates were covered with adhesive film and incubated for one hour in a humid box kept at room temperature. After incubation wells were washed with phosphate buffered saline solution (pH 7.4) containing 0.05% Tween 20 (PBS-T). All washes were repeated four times, in one wash leaving the washing buffer in contact for three minutes. Hundred µl of coating antibodies, diluted in coating buffer at 1/100, was added to each well of the ELISA plates. Plates were covered with adhesive film and incubated for two hours in a humid box at room temperature. Wells were washed three times with PBS-T. 100µl of conjugated antibodies, diluted in conjugate buffer at 1/100, was added to each well of the ELISA plates. Plates were covered with adhesive film and incubated for one hour in a humid box at room temperature. Wells were then washed three times with PBS-T. Substrate of 100µl, dissolved at 1mg/ml of substrate buffer, was added to each well containing the plant samples. ELISA plates were incubated at 37^0C for 15min and then at room temperature for the next 2 hours. ELISA plates were read using Anthos WinRead at 405nm wavelength. Samples with optical density (OD) exceeding the negative control (approximately twice) were considered as positive. All results were analysed using Genstat, General Analysis of Variance. Composition of ELISA Reagents are described in Appendix 2.

3.3 RESULTS

3.3.1 Growing-on Test

A single seedlings from the early-matured BCMV-R seeds died before emergence. Four seedlings showed malformation, stunting and blistering symptoms. From the late matured BCMV-R seeds four seedlings did not emerge. Out of the remaining seedlings, five developed symptoms. Similarly, the early-matured seeds from BCMV-U inoculated

* http://www.biorad.com

plants resulted in only one unemerged seedling while the late matured seeds resulted in five unemerged seedlings. From the late matured seeds, six developed malformation, stunting and blistering symptoms and out of the ones from the early-matured seeds, four developed typical symptoms. Symptoms are shown in Fig. 6 and 7.

Figure 6. Leaf blistering in a seedling from an early matured seed of BCMV-R inoculated plant (top) and malformed leaf of a seedling from a late matured seed of BCMV-R inoculated plant (bottom)

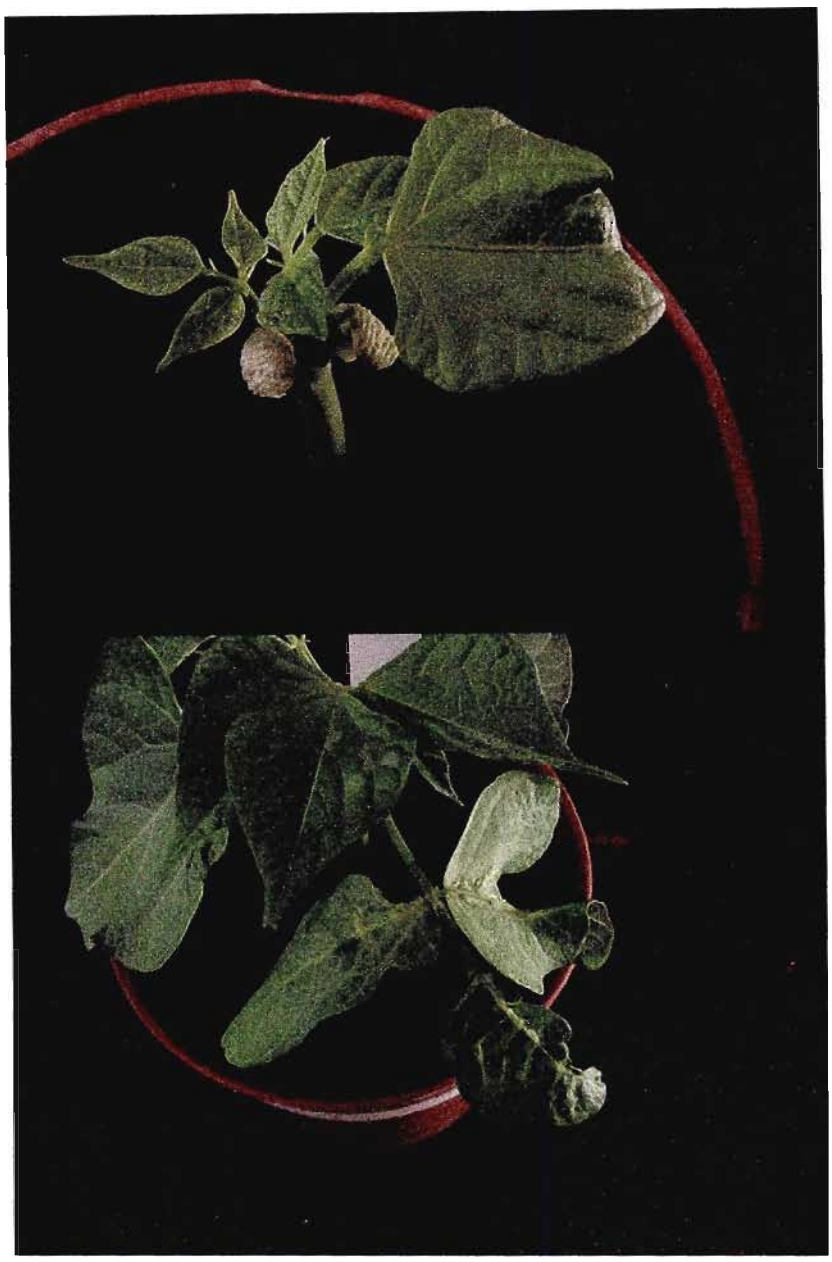


Figure 7. Stunted seedling from an early matured seed of BCMV-U inoculated plant (top) and malformed leaves of a seedling from a late matured seed of a BCMV-U inoculated plant (bottom)

3.3.2 ELISA Test

Plant samples from early-matured BCMV-R seeds gave only one positive ELISA reading. However, samples from late matured seeds gave 8 positives. Plant samples from the early-matured BCMV-U seeds gave 5 positives while those from the late matured seeds gave 8 positives. Results are shown in Table 1.

Table 1. Test results of seedlings grown from seeds harvested from virus (BCMV-R and BCMV-U) inoculated plants

Seed[2] source	No of seedlings with +ve result[1]		No of dead seedlings
	ELISA	Symptom assessment	
BCMV-RE	1^a	4^a	1^a (+ve ELISA)
BCMV-RL	8^c	5^a	4^b (2 +ve ELISA)
BCMV-UE	5^b	4^a	1^a (0 +ve ELISA)
BCMV-UL	8^c	6^b	5^b (2 +ve ELISA)
LSD	2.94	1.96	2.77
cv%	19.7	14.9	36.4

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

[2]BCMV-RE – early matured seeds harvested from plants inoculated with BCMV-R
BCMV-RL – late matured seeds harvested from plants inoculated with BCMV-R
BCMV-UE - early matured seeds harvested from plants inoculated with BCMV-U
BCMV-UL - late matured seeds harvested from plants inoculated with BCMV-U
LSD = least significant difference

Table 2. Seed transmission percent of two bean common mosaic virus isolates (BCMV-R and BCMV-U) in early and late matured seeds

Seed source	% Virus transmission[1]	
	Symptom assay	ELISA test
BCMV-RE	33[a]	8.3[a]
BCMV-RL	41.5[a]	66.7[b]
BCMV-UE	33[a]	41.7[c]
BCMV-UL	50[b]	66.7[b]
LSD	16.69	24.57
cv%	15.3	19.8

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

3.4 DISCUSSION

The results obtained show seed transmission property of the two virus isolates in the *Phaseolus vulgaris* Pannar variety (PAN 146) used in the experiment. The virus is believed to be in a high but variable proportion of the seed produced by mosaic-affected plants, depending on the bean cultivar and growth stage at which plants become infected (Morales and Castano, 1987). In their study, early-infected plants (10 days old) produced seeds with high transmission rate of the virus and seed transmission among cultivars varied from 0-57.5% depending on the virus strains. In this study, the combined result of ELISA and symptom assay tests show seed transmission of the virus isolates is quite high which reached 50% and 66.7% (from ELISA and symptom assay respectively) for late matured seeds of BCMV-U inoculated plants.

In general, the late matured seeds of the plants inoculated with the two virus isolates appear to have higher transmission rate than the early matured ones, although the difference was not significant for BCMV-R seeds. This reason may be the longer time taken by the seeds to mature and desiccate allowing the virus to multiply before the virus is inactivated in the mature seed coats as it was described by Hoch and Provvidenti

(1978). The late matured seeds also resulted in significantly greater number of unemerged seedlings than the early-matured seeds, for both virus isolates. In this case, it would be recommended by any means to avoid using late matured seeds for planting.

The ELISA test gave more positive results than the growing-on test for the three seedling groups (BCMV-RL, BCMV-UE and BCMV-UL) and less for seedlings grown from BCMV-RE. The difference could be either false positive result of ELISA or latent infection of seedlings, which cannot be detected with visual examination. However, in the later case, it appears to be false negative. As a result, when testing seeds for strict quarantine purpose, it would recommended to conduct a growing on test side by side to an ELISA test when facilities and materials are available for both.

REFERENCES

Chamberlain, E.E. 1939. Bean mosaic (Phaseolus Virus 1 of Smith, 1937). *New Zealand Journal of Science and Technology* 20: 381A-388A.

Galvez, G.E. and Cardenas-A., M. 1974. Perdidas economicas causadas por el virus del mosaico comun (BCMV). en cuatro variedades de frijol. *Proceedings of American Phytopathological Society* 121-122 (abstr.)

Hoch, H.C. and Provvidenti, R. 1978. Ultrastructural localization of bean common mosaic virus in dormant and germinating seeds of *Phaseolus vulgaris*. *Phytopathology* 68: 327-330.

Kaiser, W.J. and Mossahebi, G.H. 1974. Natural infection of mungbean by bean common mosaic virus. *Phytopathology* 64: 1209-1214.

Morales, F.J. and Castano, M. 1987. Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. *Plant Disease* 71: 51-53.

Provvidenti, R. and Braverman, S.W. 1976. Seed transmission of bean common mosaic virus in phasemy bean. *Phytopathology* 66: 1274- 1275.

Provvidenti, R. and Cobb, E.D. 1975. Seed transmission of bean common mosaic virus in tepary bean. *Plant Disease Reporter* 59: 966-969.

Reddick, D. and Stewart, V.B. 1919. Transmission of the virus of bean mosaic in seed and observations on thermal death-point of seed and virus. *Phytopathology* 9: 445-450.

Stewart, V.B. and Reddick, D. 1917. Bean mosaic. *Phytopathology* 7: 61.

Schwartz, H.F. 1989. Halo blight. In H.F. Schwartz and M.A. Pastor-Corrales, (eds), Bean production problems in the tropics, 2nd ed. Cali, Colombia.

CHAPTER FOUR

EFFECT OF BACTERIAL AND VIRAL SEED-BORNE PATHOGENS ON GERMINATION AND VIGOUR

4.1 INTRODUCTION

Seed germination and vigour are essential qualities of seed that serves as propagation material to produce next generation plants because they determine seedling emergence and establishment of plants. These qualities are affected by many factors, including pathogens. Seed-borne pathogens infect or contaminate seeds, and are transmitted to the next generation when seeds are planted. Although there is paucity of information on the effect of seed-borne pathogens on seed germination and vigour, these attributes of a seed might be affected by pathogens.

Pathogens might affect the seed in many ways. Viruses might affect the seed composition by altering some of the metabolic activities in the plant. The seed is a living organ, which respires and needs oxygen and nutrients for the process. During germination, respiration becomes fast (Copeland and McDonald, 1995) and seed pathogens might compete for oxygen as well as nutrients within the seed. Further more, toxins produced by microorganisms might create a stressful environment for germination (Campbell, 1985).

In this study, bean seeds from artificially infected plants with bacteria (*Pseudomonas syringae* pv. *phaseolicola* (Psp) *and Xanthomonas axonopodis* pv. *phaseoli* (Xap) and virus (bean common mosaic virus - BCMV) were evaluated for their performance in germination and vigour.

4.2 MATERIALS AND METHODS

4.2.1 Planting material

Bean seeds (*Phaseolus vulgaris*) of the variety PAN 146, certified to be free of *Pseudomonas syringae* pv. *syringae* (Pss), *Pseudomonas syringae* pv. *phaseolicola* (Psp) and *Xanthomonas axonopodis* pv. *phaseoli* (Xap) were used. The seed was harvested by Dr Rob Melis at Ukulinga Research Farm during the season 2002. These seeds were kept in plastic bags at a temperature of 20^0C for the duration of the experiment.

4.2.2 Plants in greenhouse

Bean seeds were planted in pots with 20cm diameter and 17.5cm height in a greenhouse. Three seeds were planted per pot and thinned 10 days later, leaving one plant per pot. Plants were grown at 25^0C and watered with an automatic sprinkler fertigation system with 3:1:3 (38) from Hortichem, Division of Ocean Agriculture.

Plants were inoculated with the pathogens. Virus inoculation procedures are those described in Chapter 3. Bacterial inoculum was prepared by growing bacteria on YDC medium as described in Chapter 2 Subsection 2.2.2. Suspensions of bacterial growths were prepared in a sterile distilled water and diluted to an optical density of 0.2-0.4 at wave length of 600mμ which represents 2 x 10^7 cells/ml for Psp and 2 x 10^8 cells/ml for Xap according to Saettler (1971). Diluted suspensions were sprayed at flowering stage when flowers were fully open according to (Marques *et al.*, 1994).

After physiological maturity (about six weeks after flowering), watering was stopped to prevent sprouting and deterioration of seeds on the plant. Pods were harvested 90 days after planting.

4.2.3 Seeds for germination and vigour test

Seeds were stored for one month to allow some degree of deterioration caused by the pathogens. Germination and vigour tests were done on the seeds with different disease

infections and on the controls. Germination tests were done according to the ISTA rules (1999) for germination. The Conductivity Test and Accelerated Aging (AA) Test were used to assess vigour according to McDonald *et al.* (2001).

The maximum weight of a seed lot for the species *P. vulgaris* is 25,000kg and a small seed lot is that which is less than or equal to 1% of the maximum lot weight (ISTA, 1999). According to ISTA (1999), the minimum submitted sample for the maximum lot is 1kg, which is 0.004% of the seed lot. In this experiment, the different seed groups that were harvested from plants inoculated with two isolates of bean common mosaic virus; from plants inoculated with common and halo blight bacteria and from healthy control plants are considered as small seed lots due to their small size. One hundred seeds each were used for germination, conductivity and accelerated aging tests. The 100 seeds represent 27-50% of the seed lots in this experiment, which is greater than the minimum proportion of sample that should be used according to ISTA (1999).

4.2.4 Germination Test

Seeds were allowed to germinate in blotter paper using the between paper (BP) method, placed in a germination chamber set at 25^0C (Fig.1). Blotter paper was kept moist by adding water at the time of counting. Germination was evaluated according to the 1999 ISTA rules and results were recorded for germinated normal seedlings daily for six days.

4.2.5 Conductivity Test

Twenty-five seeds of each seed lot were soaked in beakers containing 50ml of distilled water for 24 hrs, in four replicates. All beakers were covered with aluminium foil to reduce evaporation and dust contamination. After removing the foil and gently swirling the solution and the seeds in each beaker, the conductivity of the soak water was measured by placing a dip cell attached to a Metrohm 644 Conductometer. Measurement was done according to the Seed Technologist Training Manual (McDonald *et al.*, 2001) at the same temperature as the soaking temperature, in this case at 20^0C and results were

recorded in μs per gram weight of seed.

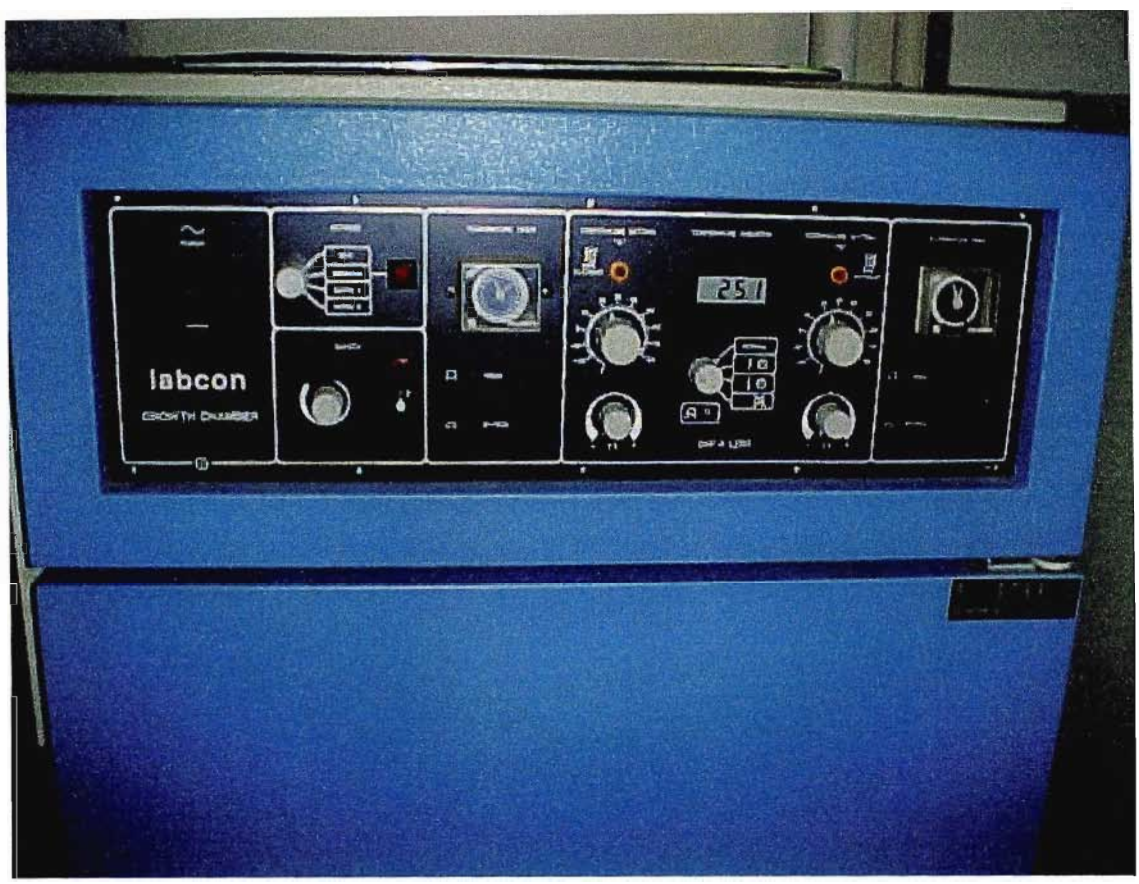


Figure 1. Germination chamber

4.2.3 Accelerated Aging Test

Twenty-five seeds were placed into a plastic mesh in a plastic container containing 100ml water to maintain near 100% RH (Fig. 2). This was done in four replicates for each seed lot. The plastic mesh was supported by a metal wire and was held 8cm above the water source. Accelerated aging was conducted according to the Seed Technologist Training Manual (McDonald *et al.*, 2001). Seeds in the containers were put in an AA chamber set at 41^0C for 48 hrs (Fig. 3).

Seeds were allowed to germinate in blotter paper by the BP method according to ISTA rules (ISTA, 1999). Seeds were placed on moist blotter paper, and the paper was rolled and put in plastic bags to maintain the moisture. Rolls were incubated in a germinating chamber at 25^0C. Germination results were recorded daily for six days.

Figure 2. Bean seeds placed on plastic mesh in a plastic container containing water and ready for accelerated aging

Figure 3. Bean seeds in containers inside an accelerated aging chamber set at 41^0C

4.3 RESULTS

4.3.1 Germination

Percent germination was calculated from the total normal seedlings, abnormal seedlings and dead seeds were not included (Fig.4). Seeds harvested from the bean common mosaic virus (BCMV) inoculated plants germinated as good as the seeds harvested from the healthy plants (Table 1). However, seeds harvested from plants inoculated with bacteria showed lower germination percentage compared to the healthy seeds. In the case of seeds from Psp inoculated plants, the difference in germination was highly significant that is 65% compared to 86% of the healthy seeds (Table 1). Seeds from Xap inoculated plants

also had low germination (76%) though statistically not significant (Table 1).

Figure 4. Normal (left) and abnormal (right) seedlings

4.3.2 Conductivity

The electrolytic conductivity of the seed soak was significantly lower (542.5µs) for seeds harvested from the BCMV-R inoculated plants as compared to that of the healthy plants (605.0µs) (Table 1). In contrast, electrolytic conductivity of seed soak for seeds from Xap inoculated plants was significantly higher than that of the control, which was 662.5µs (Table 1). Seed soaks for seeds from BCMV-U and Psp inoculated plants also showed higher electrolytic conductivity (637.5µs and 635.0µs respectively) although it was not statistically significant (Table 1).

4.3.3 Accelerated Aging (AA)

Germination of accelerated aged seeds from the Psp inoculated plants decreased to more than half of that before aging (from 65% to 32%). Seeds from the BCMV inoculated plants did not show significant difference in germination compared to the healthy seeds even after aging. In contrast, seeds from Xap inoculated plants showed significantly lower germination (52%) compared to the healthy seeds (80%) after aging (Table 2).

Table 1. Germination percentage of seeds harvested from *Pseudomonas syringae* pv. *phaseolicola* (Psp), *Xanthomonas axonopodis* pv. *phaseoli* (Xap), or bean common mosaic virus (isolates BCMV-R and BCMV-U) inoculated plants and clean control seeds (C) and electrolytic conductivity of seed soaks

Seed group	Germination %[1]	Conductivity in µs[1]
C	86[a]	605.0[a]
Psp	65[b]	635.0[cd]
Xap	76[a]	662.5[d]
BCMV-R	80[a]	542.5[e]
BCMV-U	83[a]	637.5[cd]
LSD	10.38	33.53
cv%	8.8	3.6

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

Table 2. Germination percentage of seeds harvested from *Pseudomonas syringae* pv. *phaseolicola* (Psp), *Xanthomonas axonopodis* pv. *phaseoli* (Xap), bean common mosaic virus (isolates BCMV-R and BCMV-U) inoculated plants and control seeds (C) after accelerated aging

Seed group	Germination %[1]
C	80[a]
Psp	32[b]
Xap	52[c]
BCMV-R	71[a]
BCMV-U	70[a]
LSD	10.98
cv%	11.9

LSD= least significant difference at 5% level

[1] Letters which are the same do not differ at P=0.05

4.4 DISCUSSION

Seed germination is known to be affected by many factors including pathogens. Warham (1990) reported reduction in wheat seed germination due to the fungus *Tilletia indica*, the causal organism of Karnal bunt. In this study, there was reduced seed germination of sugar bean seeds harvested from plants inoculated with the bacteria Psp and Xap (the causal organisms of bean halo and common blights respectively).

Seeds from Psp inoculated plants exhibited significantly lower germination than any of the other seeds in the test. Although the concentration of bacteria inoculated to the plants (on their flowers) was lower for Psp than for Xap, reduction of germination was higher in seeds from Psp inoculated plants. This could be due to the faster growth rate of the bacteria Psp than Xap. The greater reduction in germination due to these pathogens (Psp and Xap) than the bean common mosaic virus (BCMV) isolates was probably the result of competition for oxygen with the seeds by the gram-negative bacteria- Psp and Xap - as it was suggested by Campbell (1985). Seeds need adequate levels of oxygen for respiration during germination. Germination responses that are not directly affected by increased levels of respiration are also enhanced due to the ability of oxygen to decrease some levels of inhibitors (Copeland and McDonald, 1995). The bacteria might compete for the storage product metabolites, which are necessary to provide energy for the growth of the embryonic axis during the germination process. This could explain why there were many abnormal seedlings when seeds harvested from plants inoculated with these bacteria were allowed to germinate. For seeds obtained from Psp inoculated plants, there was an average of one dead seed per replicate, both in the germination test and accelerated aging test. In this case, the bacteria might have multiplied and reached a concentration that would not allow the seed to utilize the nutrient sources during imbibition. The germination temperature was 25^0C, which is favourable for the bacteria, and Psp has faster growth rate than Xap. It is also possible that bacterial toxins were also involved in reducing the germination of the seeds.

The electrolytic conductivity of seeds harvested from Xap inoculated plants was

significantly higher than that for the seeds which were harvested from healthy plants while that of seeds harvested from the BCMV-R inoculated plants was significantly lower than that for the control seeds. However, for seeds harvested from Psp and BCMV-U inoculated plants although the conductivity was greater than the control, the difference was not significant.

Although the germination percent seeds harvested from Xap inoculated plants was lower than that of the healthy seeds, it was slightly less than the significant level. However, when seeds were allowed to germinate after aging, Xap seeds showed significantly lower germination than aged seeds from healthy controls (52% compared to 80% for healthy seeds). The result from the conductivity test also showed that seeds from Xap inoculated plants had higher conductivity compared to the control seeds. Higher electrolyte leakage reveals poorer ability of the seed to re-establish its membrane integrity. Powell (1988) stated that the integrity of cell membranes could be considered the fundamental cause of differences in seed vigour. Similarly, the low vigour of seeds harvested from Xap inoculated plants could be due to poor membrane integrity, caused by the bacteria. In the case of seeds harvested from Psp inoculated plants, germination percent was less than half of that for the healthy seeds (32% for Psp compared to 80% for healthy seeds) and it was lowered to about half of that before aging (65% before aging). As the conductivity of these seeds was not significantly different from that of the control seeds, the low germination and vigour was probably due to competition of the bacteria with the seed for nutrient and oxygen, rather than poor cell membrane integrity. Accelerated aging test is an indirect measure of seed vigour, which is related to emergence. So the results of reduced seed germination after AA for seeds from Psp and Xap inoculated plants agree with findings from Vennette (1985), who found reduced emergence of bean seeds infected with Psp and Pss.

In the AA test, seeds from plants inoculated with the two isolates of the virus BCMV and seeds from healthy plants showed similar germination to that before aging. The results from the conductivity test showed that seeds from BCMV-R inoculated plants had

significantly lower conductivity than the control while there was no significant difference in that of the seeds from BCMV-U inoculated plants and the control seeds. This shows that seeds from BCMV inoculated plants might have better cell membrane integrity (in the case of BCMV-R) or similar to that of healthy ones (in the case of BCMV-U). In this experiment, there was no significant difference in seed germination and vigour of seeds from BCMV inoculated plants and seeds form healthy plants. In contrast, Abd-El-Mageed (1995) reported that the percentage germination of seeds infected by BCMV was greater than that of uninfected seeds of two cultivars (Swiss Blanc and Contender) of *Phaseolus vulgaris*. This could be due to use of a different cultivar (PAN 146) in this experiment or use of different strains of BCMV.

The bacterial seed-borne pathogens Psp and Xap in this study affected seed germination and vigour or vigour only respectively. Planting infected seeds in areas where environmental conditions are unfavourable for the bacteria might reduce disease severity but germination and vigour of seeds could be affected. In contrast, neither germination nor vigour was affected by BCMV. Therefore when planting BCMV infected seeds, most of the seeds will germinate and develop into a full plant, increasing the disease spread and severity under favourable conditions to the virus. For these reasons it would be recommended to avoid Psp, Xap and BCMV infected seeds as a planting material.

REFERENCES

Abd-El-Mageed, M.H. 1995. Inhibitory effect of some fungal growth products on bean common mosaic virus infectivity and seed germination of BCMV-infected bean plants. *Annals of Agricultural Science* 33: 1291-1305.

Campbell, R. 1985. Plant microbiology. Arnold, London.

Copeland, L.O. and McDonald, M.B. 1995. Principles of Seed Science and Technology, 3rd ed. Chapman and Hall, New York.

ISTA. 1999. International rules for seed testing. Supplement to Seed Science and Technology 27: 155-1999.

Marques-As-Dos, Parente-PMG, Machado-Foc, Santana-CR and Dos-Marques-As. 1994. Screening of inoculation methods to produce bean seeds contaminated with *Xanthomonas campestris* pv. *phaseoli* for experimental purposes. *Fitopathologia-Brasileria* 19: 178-182.

McDonald, M.B., Gutormson, T. and Turnipseed, B. 2001. Eds. Seed technologist training manual. Society of Commercial Seed Technologists, U.S.A.

Powell, A.A. 1988. Seed vigour and field establishment. *Advances in research and technology of seeds* 11: 29-61.

Saettler, A.W. 1971. Seedling injection as an aid in identifying bean blight bacteria. *Plant Disease Report* 55: 703-706.

Vennette, J.R. 1985. Reduction of bean seed emergence by vacuum infiltration with bacterial pathogens. *Phytopathology* 75: 385.

Warham, E.J. 1990. Effect of *Tilletia indica* infection on viability, germination, and

vigour of wheat seed. *Plant Disease* 74: 30-132.

CHAPTER FIVE

THE EFFECT OF BEAN VIRUSES ON BEAN WEEVIL INFESTATION

5.1 INTRODUCTION

Dry bean seeds could be badly damaged by insect pests during storage. The bean weevil (*Acanthoscelides obtectus* (Say)) is one of the two major post harvest pests that attack dry beans worldwide (Skaife, 1918; Hoffmann *et al.*, 1962, cited in Multon, 1988).

Skaife (1918) stated that the adult weevils appeared in the field during January, when the first crop of beans was ripening and continued to be among the bean plants throughout the summer. Multon (1988) also reported that at higher temperature and sunny weather the adults migrate from the stores towards the growing crops in the field where mating occurs.

The female deposits her eggs inside the ripe dry pods. She first gnaws a slit about one millimetre long in the ventral suture of the pod and drops her eggs loosely inside the pod by inserting her ovipositor in the slit (Skaife, 1918). The eggs in infested pods hatch after several days (about ten days) and the larva bore into the dry seeds, where they reach maturity after three to four weeks and the pupal stage lasts about eight to eleven days in summer (Skaife, 1918). Thus when infested seeds are harvested, the beetles can appear in the stored seeds after six weeks in warm weather.

Several control measures are suggested in the literature. These include cold storage (Jones, 2002, Karapetyan, 1983), breeding for resistance (Ishimoto, 2000, Jones, 2002), coating seeds with oils (Skaife, 1918, Jones, 2002), chemical control (Bogs, 1981, Kr''steva, 1982) and biocontrol using *Pediculoides (Heteropus) ventricosus* Newport (Skaife, 1918) and *Aspergillus ochraceus* (Gafurova and Kurbatskaya, 1980).

In this study, common bean (*Phaseolus vulgaris*) plants were grown in a greenhouse. Three groups of plants were grown: 1) plants inoculated with strains of bean common

mosaic virus (BCMV), 2) plants inoculated with *Pseudomonas syringae pv. phaseolicola* (causal organism of bean halo blight) 3) uninoculated plants (control). The seeds from these plants were harvested at the same time and stored under similar storage conditions as described under the materials and methods section below. Bean weevil infestation was observed in stored seeds after six weeks. The level of damage caused by the bean weevil on the different seed groups that were harvested from control, Psp and BCMV inoculated plants was evaluated. Germination of undamaged seeds from the infested lots was also evaluated.

5.2 MATERIALS AND METHODS

5.2.1 Planting material, plants in the greenhouse, inoculum and inoculation

The seed source, greenhouse conditions, inoculum and inoculation procedures were similar to those described in previous chapters, except that the Psp and BCMV-R isolates were different. In this case, the inoculum for Psp was Sp75 and for BCMV-R was BCMV93/0032.

5.2.2 Harvest and storage

Plants were left in the greenhouse until the pods mature and the leaves fall. About 90 days after planting the pods were harvested and left to dry in the air at a room temperature for ten days, after which they were packed in paper bags inside plastic bags for about 30 days at room temperature. The seed groups harvested from the control (uninoculated plants), bacteria and virus inoculated plants were handled in the same manner, packed separately and stored under the same temperature and moisture conditions.

5.2.6 Weevil infestation

The seeds were harvested from the green house for an experiment that was planned to see the effect of the diseases on seed germination and vigour. However, after storage the seeds harvested from the control and Psp inoculated plants were found to be seriously

infested with the bean weevil (*Acanthoscelides obtectus* Say.) (Fig.1 and Fig.2).

5.2.7 Evaluation of weevil damage

Seeds with a hole or holes were classed as damaged and the total number of damaged seeds were counted for each batch of seeds. The number of holes were also counted in each of the damaged seeds and seeds were rated according to the number of holes. Damage on seeds with 1-2 holes was considered as slight, 3-4 holes as moderate and >5 holes as heavy. The levels of weevil damage for each seed lot were statistically analysed using pairwise T-test comparisons, using S.A.S. software.

5.2.8 Seed germination

Undamaged seeds were allowed to germinate in petri dishes in between two moistened filter papers by the between paper (BP) method (ISTA, 1999), placed in a germination chamber set at 25^0C. Seeds germinated in four replicates, 25 seeds per replicate. Filter papers were kept moist by adding water daily. Germination was evaluated according to the ISTA rules (ISTA, 1999) and the numbers of normal, abnormal seedlings and dead seeds were counted and recorded daily for six days. Results were analysed using Genstat, General Analysis of Variance.

5.3 RESULTS

5.3.1 Level of damage

Seeds harvested from the BCMV-U had a little infestation and seeds from the BCMV-R inoculated plants had no infestation (Tables 1). Weevil infestation of the control seeds was significantly higher at $P \geq 1\%$ compared to both BCMV-U and BCMV-R infected batch of seeds (Table 2). Infestation of Psp infected batch of seeds was also significantly higher at $P \geq 5\%$ compared to the BCMV infected batch of seeds. However, there was no significant difference between infestation levels of the control and Psp infected batch of seeds as well as between BCMV-U and BCMV-R infected batch of seeds (Table 2).

5.3.2 Seed Germination

Undamaged seeds from the BCMV infected plants and the control seeds of the infested seed lots did not show significant difference in their germination. Seeds from Psp infected plants resulted in a significantly low germination percentage (Table 3). For the sake of comparison, results from another experiment (Chapter 4, germination of seeds from uninfested seed lots) are included here (Table 4).

Figure 1. Bean seeds with holes made by the bean weevil (*Acanthoscelides obtectus* (Say))

Figure 2. The bean weevil (*Acanthoscelides obtectus* (Say)) on bean seed

Table 1. Levels of damage caused by the bean weevil on seeds infected with halo blight bacteria (Psp), two bean common mosaic virus isolates (BCMV-R and BCMV-U) and Control seeds

Seed group	Level of damage	No. of seeds	% of damage
Control	Undamaged	405	75.3
	Slight	66	12.3
	Moderate	33	6.1
	Heavy	34	6.3
	(Total damaged)	133	24.7
Psp	Undamaged	371	88.3
	Slight	24	5.7
	Moderate	18	4.2
	Heavy	7	1.7
	(Total damaged)	49	11.6
BCMV-U	Undamaged	321	98.8
	Slight	4	1.2
	Moderate	0	0
	Heavy	0	0
	(Total damaged)	4	1.2
BCMV-R	Undamaged	219	100
	Slight	0	0
	Moderate	0	0
	Heavy	0	0
	(Total damaged)	0	0

Undamaged = no holes
Slight =1-2 holes
Moderate =3-4 holes
Heavy = >5 holes

Table 2. A pairwise comparison of the different seed lots infected with halo blight bacteria (Psp), bean common mosaic viruses (BCMV-U and BCMV-R) and uninoculated control seeds for damage caused by bean weevil using T-test

Seed group	Psp	BCMV-U	BCMV-R
Control	0.1361^{NS}	0.0195^{**}	0.0155^{**}
Psp		0.0483^{*}	0.0295^{*}
BCMV-U			0.3739^{NS}

^{NS} Non significant
* Significant at 5%
** Highly significant at 1%

Table 3. Germination of undamaged seeds from seed lots infested with bean weevil and seeds infected with halo blight bacteria (Psp), two bean common mosaic virus isolates (BCMV-R and BCMV-U) and disease free control seeds

Seed group	% Germination[1]
Control	79^{a}
Psp	58^{b}
BCMV-R	78.7^{a}
BCMV-U	68^{ab}
LSD (5% level)	13.47
cv%	10.8

LSD= least significant difference at 5% level
[1] Letters which are the same do not differ at P=0.05

Table 4. Germination of seeds infected with halo blight bacteria (Psp), two bean common mosaic virus isolates (BCMV-R and BCMV-U) and disease free control seeds from seed lots uninfested with the bean weevil

Seed group	% Germination[1]
Control	86^{a}
Psp	65^{b}
BCMV-R	80^{a}
BCMV-U	83^{a}
LSD (5% level)	10.38
cv%	8.8

LSD= least significant difference at 5% level
[1] Letters which are the same do not differ at P=0.05

5.4 DISCUSSION

The level of infestation by the bean weevil was highest in the control seeds, which were not subjected to a disease. Among the seeds from infected plants, seeds that were harvested from plants inoculated with the halo blight bacteria showed a high level of damage. The seeds harvested from the bean common mosaic inoculated plants showed little infestation by the bean weevil. Other researchers have found that bean seeds with lower starch content, thicker cuticle and testa, and higher tannin content were found to be more resistant to the insect (Ciepielewska *et al.*, 1993; Ciepielewska and Fornal, 1993; Ciepielewska and Fornal, 1994). Virus infection alters many aspects of plant metabolism. Brennan and Leone (1969) postulated that tobacco mosaic virus (TMV) altered the carbohydrate and nitrogen metabolism of the plant, which was found to be protected against ozone (O_3) injury. Davis and Smith (1974) also found that BCMV inoculated bean leaves had less O_3 injury than uninoculated ones. The resistance to infestation by the bean weevil observed in this experiment could be due to the alteration of some of the plant metabolism aspects such as contents of starch and tannin and characteristics of the seed coat, cuticle and testa. Further confirmation studies and investigating the mechanisms involved might provide important information to the seed breeder on the development of resistance against the pest. If this is the case, it might also be possible to use mild strains of BCMV to protect bean seeds against this pest. Mani *et al.* (1987) found that mild strains of citrus tristeza virus (CTV) could control citrus nematode. Stem-pitting effects of citrus caused by the virulent strains of CTV are also satisfactorily controlled by mild strains of the virus under commercial conditions (Muller, 1980).

The difference of germination percentage of seeds harvested from the bean common mosaic virus (BCMV) inoculated plants and the control was not statistically significant. However, germination of seeds harvested from the bacterium (Psp) inoculated plants was significantly lower. Similar results were obtained in another experiment (with no weevil infestation) for the germination of seeds from Psp and BCMV inoculated plants and an uninfected control. The reduced germination percent for undamaged seeds from Psp

inoculated plants in the infested lot appears to be due to the bacteria. Undamaged seeds from weevil infested seed lots may not be affected in their germinating capacity.

This observation needs further study by artificially infesting bean seeds harvested from BCMV inoculated plants, with the bean weevil. If results are confirmed it might provide important information to the seed breeder, seed technologist and entomologist so that they would work together to bring a solution to the pest problem.

REFERENCES

Bogs, D. 1981. The control of the bean weevil (*Acanthoscelides obtectus* Say) with hydrogen phosphide. *Nachrichtenblatt fur den Pflanzenschutz in der DDR*, 35, 10:210.

Brennan, E. and Leone, I.A. 1969. Suppression of ozone toxicity symptoms in virus-infected tobacco. *Phytopathology* 59:263-264.

Ciepielewska, D. and Fornal, L. 1993. The preference of the bean weevil (*Acanthoscelides obtectus* Say, Coleoptera, Bruchidae). *Polskie Pismo Entomologiczne* 62 : 153.

Ciepielewska, D. Kordan, B. and Fornal, L. 1993. The preference of the bean weevil (*Acanthoscelides obtectus* Say, Coleoptera, Bruchidae). Part I. Morphological and physiological characters of bean seeds of various cultivars and the development of the insect pest. *Polskie Pismo Entomologiczne* 62: 145-152.

Ciepielewska, D. and Fornal, L. 1994. Natural resistance of Phaseolus seed cultivars to the infestation by the bean weevil (*Acanthoscelides obtectus* Say). *Acta Academiae Agriculturae ac Technicae Olstenensis, Agricultura* 59: 97-105.

Davis, D.D. and Smith, S.H. 1974. Reduction of ozone-sensitivity of Pinto bean by bean common mosaic virus. *Phytopathology* 64:383-385.

Gafurova, V.L. and Kurbatskaya, Z.A. 1980. The influence of endotoxins of entomopathogenic fungi on their virulence. *Mikrobiologicheskii Zhurnal* 42: 591-595.

Hall, D.W. 1970. Handling and storage of food grains in tropical and subtropical areas. FAO, Rome. pp92-93.

Hoffmann, A., Labeyrie, V. and Balachowsky, A.S. 1962. Famille des Bruchidae. In A.S. Balachowsky, (ed). Traite d'entomologie appliquee a l'agriculture. Masson, Paris, 1,

434-494.

Ishimoto, M. 2000. Evaluation and use of wild *Phaseolus vulgaris* species in breeding. The Seventh Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan, International Workshop on Genetic Resources, Ibaraki, Japan, 13-15 October, 1999: Part 1, wild legumes. pp. 183-190.

ISTA. 1999. International rules for seed testing. Supplement to Seed Science and Technology 27: 155-1999.

Jones, A.L. 2002. Phaseolus bean: post-harvest operations. Available at: http://www.fao.org/inpho/compend/text/ch04-03.htm

Karapetyan, A.P. 1983. The ecology and harmfulness of the bean weevil *Acanthoscelides Obtectus* (Say) in the conditions of the Armenian SSR. *Biologicheskii Zhurnal Armenii* 36: 647-651.

Kr''steva, L. 1982. The bean weevil and its control. *Rastitelna Zashchita* 30: 47-48.

Mani, A., Naidu, P.H. and Dakshinamurti, V. 1987. Influence of citrus nematode, *Tylenchulus semipenetrans* on growth of Kagzi lime and its interaction with a mild strain of tristeza virus. *Indian Phytopathology* 40: 27-29.

Marques-As-Dos, Parente-PMG, Machado-Foc, Santana-CR, and Dos-Marques-As. 1994. Screening of inoculation methods to produce bean seeds contaminated with *Xanthomonas campestris* pv. *phaseoli* for experimental purposes. *Fitopathologia Brasileria* 19: 178-182.

Muller, G.W. 1980. Use of mild strains of citrus tristeza virus (CTV) to reestablish commercial production of 'Pera' sweet orange in Sao Paulo, Brazil. *Proceedings of the Florida State Horticultural Society* 93: 62-64.

Multon, J.L. 1988. Preservation and storage of grains, seeds and their by-products: Cereals, oilseeds, pulses and animal feed. Lavoisier, USA. pp378-380

Skaife, S.H. 1918. Pea and Bean Weevils. *Bulletin of the Union of South Africa, Department of Agriculture* 12: 13-20.

APPENDICES

APPENDIX 1

Guide for ISTA/ISHI Comparative Test for *Xanthomonas axonopodis* pv. *phaseoli* 2002 and composition of media

This appendix contains the materials, methods and procedures used for preparing media for detection of bacteria. Part I of Appendix 1 is the guide for ISTA/ISHI Comparative Test, *Xanthomonas axonopodis* pv. *phaseoli* 2002 edited by Jim Sheppard. Part II of this Appendix is taken from ISTA/ISHI Comparative Test, *Xanthomonas axonopodis* pv. *phaseoli* 1999 prepared by J.W. Sheppard and H. Koenraadt. Part III is from phytobacteriology manual by Goszczynska, *et al.* 2000 (Goszczynska, T.; Serfontein, *J. J.* and Serfontein, S. 2000. A SAFRINET MANUAL FOR PHYTOBACTERIOLOGY, Introduction to Practical Phytobacteriology. SAFRINET, Pretoria.). Part IV is from Young *et al.* (1978) (Young, Dye and Wilkie. 1978. Bean Halo Blight in: ISTA Handbook for Seed Health Testing. Working Sheet No 66.)

Part I
ISTA/ISHI Comparative Test: *Xanthomonas axonopodis* pv. *phaseoli*

SUMMARY

Object: To determine suitability of dilution plating semi-selective media for detection of *Xanthomonas axonopodis* pv *phaseoli* in bean seed.

Background: A number of semi-selective media have been reported for the isolation of pathovars of *Xanthomonas axonopodis.* These media depend on the ability of Xanthomonas to degrade starch, casein or Tween, leaving clear zones of hydrolysis around the colony. Previous comparative tests (1995-1998) have evaluated several semi-selective media for use in the detection of *Xanthomonas axonopodis* pv. *phaseoli* (Xap) in bean seed. Two media XCPl and MT have been chosen for their ease of use and selectivity for Xap. These will be evaluated for suitability in routine seed health testing of

bean seed for the presence of *Xanthomonas axonopodis* pv. *phaseoli.* The ability of laboratories to recognize this pathogen on the various media will also be evaluated.

XCP 1 *Xanthomonas axonopodis* pv. *phaseoli* media adapted from (McGuire, et al. Plant Disease 1986 70:887- 891)

MT Milk-Tween adapted from BBD by Goszczynska and Serfontein, J. Microbiological Methods: 1998 32: 65- 72.

Method:

Samples

5 lots (5 replicates each) representing various infection levels 1 high (all sub-samples positive) 3 moderate (1-4 sub-samples positive) 1 low (This sample has been demonstrated to have very low levels of Xap)

Reference Cultures:

Working cultures obtained from

ATCC 19315 (fuscans strain)

ATCC 9563 (non-fuscans strain)

Extraction & Plating

Overnight seed soak at 5°C followed by dilution plating of 1 00 µl aliquot for each of 4 dilution levels for each seed extract (10^{0}-10^{-3}) onto 2 plates for each of 2 media (XCP1, MT).

Incubation:

3-5 days @ 27°C (4 days optimum)

Recording Results

Following incubation count colonies

(a) in each plate containing between 15 and 150 Xap suspect colony forming units and
(b) total non-Xap colonies for the same plates.

Identification:

Identity of selected isolates confirmed by a YDC screen and pathogenicity test.

DETECTION OF COMMON BACTERIAL BLIGHT IN FIELD BEAN Crop: *Phaseolus vulgaris*

Pathogen: *Xanthomonas axonopodis pv. phaseoli* (Common Blight)

Syn. *Xanthomonas campestris* pv. *phaseoli* (*Xanthomonas phaseoli*)

Prepared by: J. W. Sheppard[1] and Harrie Koenraadt[2]

[1]Canadian Food Inspection Agency, Central Seed Laboratory, Bldg #22, C. E. F., Ottawa, Ontario Canada KIA OC6 (email: sheppardj@inspection.gc.ca)

[2] P.O. Box 40,2370 AA Roelofarendsveen, The Netherlands (email: h.koenraadt@naktuinbouw.nl)

BACKGROUND

Previous comparative tests (1995-1998) have evaluated several semi-selective media for

use in the detection of *Xanthomonas axonopodis* pv *phaseoli* (Xap) in bean seed. Two media XCPI and MT have been chosen for their ease of use and selectivity for Xap. Both media rely on the ability of Xap to hydrolyse of starch or casein. PTS has been included in this and other tests as a reference medium.

SAFETY PRECAUTIONS

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving and weighing out of antibiotics. It is assumed that persons familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique are carrying out this procedure in microbiological laboratory. Dispose of all waste materials in an appropriate way (e.g. autoclave, disinfect) and in accordance with local safety regulations.

MATERIALS

Reference Cultures

Reference cultures of known pathogenicity are included in this test kit.

A: Isolate 1	B: Isolate 2

Media, Antibiotics, Dyes and other Reagents

To avoid difficulties in obtaining the various antibiotics we have prepared all the materials. Each test kit contains sufficient material to prepare 5 liters of each medium.

1. Seed Samples

This test kit will contain 20 seed packets each containing 1000 seeds each. These packets represent sub-samples drawn from seed lots test and found to contain (a) a high level of infection and high numbers of other organisms; (b) seed from moderate to low levels of infection and (c) a lot which has been demonstrated to have very low levels of Xap. Each packet is uniquely numbered and the relationship of each sample to others in the set is known only to the test organizer(s).

2. Other Materials and Equipment

- 20 - 1-liter beakers for soaking seeds

- Sterile distilled water (600 ml/sample)

- Tubes or bottles containing 4.5 ml sterile nutrient broth for dilutions - Pipettes or pipettors (check accuracy and precision regularly) - Sterile pipette tips

- 1 ml hypodermic syringes for pathogenicity tests or sterile toothpicks - Incubator at 27°C

- Susceptible bean plants for pathogenicity test (seed supplied)

Preparation of samples

It is vital to exclude any possibility of cross-contamination between seed samples; it is therefore essential to disinfect all surfaces, containers, hands, etc. both before and after handling each sample. This can achieved by swabbing/spraying equipment and gloved hands with 70% ethanol.

METHODS

1 *Extraction*

Suspend seeds in sterile distilled water 600 ml per 1000 seeds.

1.1 Prepare 10 fold dilution series (10°, 10^{-1}, 10^{-2}, 10^{-3}).

1.2 Spread 100µl of the seed leachate onto two plates for each dilution for each media under evaluation.

1.3 Incubate plates at 28°C. 3-5 days (4 days optimum).

2 *Positive control (culture)*

2.1 Prepare a suspension of two known strains of Xap in sterile nutrient broth.

2.2 Dilute sufficiently to obtain dilutions containing approx. 10^2 to 10^4 cfu/ml.

2.3 This may require up to seven ten-fold dilutions from a turbid suspension (ca. 10^8 cfu/ml).

2.4 Pipette 100µl of appropriate dilutions onto plates of test media and spread over the surface with a bent glass rod.

2.5 Incubate control plates with sample plates.

3 *Evaluation*

3.1 Colony characteristics

MT: *Xanthomonas* colonies are yellow mucoid, convex, distinguished by two zones of hydrolysis: (1) a large clear zone of casein hydrolysis and (2) a smaller milky zone of

Tween 80 lysis. Fuscans strains of Xap are distinguished by distinct brown pigmentation.

XCP1: Colonies are yellow mucoid, convex surrounded by a clear zone of starch hydrolysis. Fuscans strains of Xap are distinguished by distinct brown pigmentation.

4 *Examination of Plates*

4.1 Examine and record observations after 3-5 days (4 days optimum).

4.2 Select plates containing between 15 and 150 suspect colonies.

4.3 Record total number of colonies for each sample on the appropriate worksheet or on spreadsheet provided on the diskette as follows:

(A) Suspect Xap total cfu

(B) Non-suspect Xap total cfu

5 *YDC Screen*

5.1 Sub-culture suspected colonies to sectored plates of YDC. To avoid the potential for cross-contamination of isolates, use a new sectored plate for each sub-sample. The precise numbers of colonies sub-cultured will depend on the number of suspect colonies, but 5 seem to be an accepted number.

5.2 Sub-culture positive control isolate to a sectored plate for comparison.

5.3 Incubate sectored plates for 24-48h at 27°C.

5.4 Compare appearance of growth with positive control. On YDC Xap colonies are bright yellow and mucoid/fluidal.

6 *Confirmation / Pathogenicity Test*

The purpose of this test is to evaluate the efficacy of various semi-selective media for isolation of the pathogen from bean seed. Confirmation of the identity and pathogenicity of isolates from the various media is not intended as part of this comparative test. Collaborators are free to use the method of choice for their laboratory for this purpose, however; for the sake of uniformity the following procedures must be included.

Seedling inoculation (Based on: Saettler, A. W. 1971, Seedling injection as an aid to identifying bean blight bacteria. Plant Disease Reporter. 55:703-706).

6.1 Plant 3 seeds 2 cm deep in potting soil in a 10 cm pot.

6.2 Incubate plants in a greenhouse or phytotron at 25°C.

6.3 Select max 5 typical isolates from YDC sectored plates for each sample/media combination for use in inoculation test.

6.4 Inoculate seedlings at the first true leaf stage (first leaves unfolded and first trifoliate leaf is partially open).

6.5 Water plants thoroughly approx 2 hours before inoculation.

6.6 Dip sterile needle or toothpick into a distinct colony.

6.7 Inoculate the seedling by stabbing the needle or toothpick through the primary node at an angle of ca 45°. Stop stabbing as the needle emerges from the opposite side of the node.

6.8 Turn needle or toothpick slightly while withdrawing to release bacteria. Support the seedling with one hand during the process. Inoculate 2 seedlings per pot in this manner dipping the needle into the colony before inoculation of each seedling. Label the uninoculated seedling in each pot as non-treated seedling.

6.9 Prepare pots containing positive (reference cultures) and negative (sterile water) and

uninoculated toothpick or needle controls in a similar manner.

6.10 Put filter paper on the bottom of a tray. Saturate filter paper with water. Transfer pots to the tray. Cover the tray with a plastic bag or a transparent lid for 2 days to ensure a high relative humidity.

6.11 Incubate plants in the greenhouse or phytotron for 2 weeks. Remove plastic after 2 days.

6.12 Evaluate symptoms of inoculated seedlings 7 and 14 days after inoculation. Record observations on the pathogenicity test record sheet.

General methods (common to many test procedures)

1. *Numbering of samples/isolates*

Each seed lot/sample has been allocated a unique sample number and assigned to one of the two media under test e.g. 123 XCPI. Each sub-sample of a lot should also be given number, e.g. 123 XCPI/1 (seed lot no 123 media XCPI, sub-sample 1). Keep the sub-sample numbers within a seed lot unique, i.e. there should only be one 123 XCPI/1 regardless of the test date. Each colony sub-cultured from a sub-sample should also be given a number and should be unique within the sub-sample, regardless dilution. e.g. 123 XCPI/1.1 (lot 123, media XCPI, sub sample 1, colony 1) and colonies 1-3 could come from one dilution and colonies 4-6 could come from another dilution. Record where they have come from by writing the colony numbers on the dilution plate recording sheet.

2. *Preparation of ten-fold dilution series*

Each dilution should be prepared by pipetting 0.5 ml from a well-mixed seed extract or previous dilution into a universal bottle (screw cap bottle) containing 4.5 ml of sterile diluent and then vortexing to mix prior to the next dilution step. A new sterile pipette tip

should be used for each dilution step.

3. *Plating of dilutions.*

This should be done as soon as possible after dilutions have been prepared (within 20 min). Working from the highest (most dilute) dilution to the undiluted extract, 0.1 ml is pipetted onto the surface of a surface-dry, labelled agar plate. The liquid should then be spread evenly over the entire surface of the plate with a bent glass rod. If care is taken to work from the highest to lowest dilution (or undiluted extract) a single pipette tip and a single bent glass rod can be used for each sample. Ensure that all liquid has been absorbed by the agar before inverting and incubating plates. If necessary allow plates to dry under a sterile airflow in a microbiological safety cabinet.

4. *Recording of dilution plates*

Record the results for all dilution plates on the recording sheets. The most accurate estimate of bacterial numbers should be obtained from spread plates with between 30 and 300 colonies. However this may be further complicated depending on the relative numbers of suspect pathogen and other colonies. In order to minimise effort, start recording with the highest dilution (most dilute) and count the number of suspect and the number of other colonies; record as >st= (sterile) if there are no colonies on the plate. Go on to the next highest dilution. If it is obvious that the number of colonies on a plate greatly exceeds 300 there is little value in trying to make a precise count if a more reliable count has already been obtained from a more dilute plate, in which case it is sufficient to record the number of colonies as >m= (many) if they are still separate or >c= (confluent) if they have run together. Thus, each box on the dilution plate recording sheet should contain a number, >st=, >m=, or >C=. At a given dilution it would be quite reasonable to have a different type of record for suspect colonies and others, e.g. if the numbers of saprophytes is high and pathogen low at a particular dilution the number of others could be recorded as >m= and the number of suspect as >5=

5. *Sectored Plates*

Using a laboratory marker pen draw lines on the base of a standard 9 cm agar plate to divide it into six sectors. Using a sterile microbiological loop sub-culture single colonies from dilution plates and make a single zigzagged streak within a single sector on the plate. Take care to leave sufficient space between each isolate to ensure the growth does not coalesce these six suspect colonies can be sub-cultured to each sectored plate. If any doubt about the purity of sub-cultured isolates, these should be further streaked out on whole plates by conventional means.

Preparation and composition of Media

MT (MT_{new})

Compound	Source	Cat #	gms/L
A			
Proteose peptone #3	Difco	0122-17-4	10
$CaCl_2$	BDH	27588 4T	0.25
Tyrosine	Sigma	T-1145	0.5
Agar	Difco	0140-01	15
Distilled water			500
B			
Skim milk powder	Oxoid	L31	10
Distilled water			500
C			
Tween 80	Fisher	T164 500	10ml
D			
Nystatine	Duchefa	N0138	40mg
Cephalexin	Sigma	C-4895	80mg
Vancomycin	Sigma	V-2002	10mg

Preparation

1. Weigh out all ingredients in section A into a suitable container

2. Add 500 ml of distilled water

3. Dissolve ingredients

4. Dissolve skim milk powder separately in 500 ml water

5. Separately prepare 10 ml Tween 80

6. Sterilize, preparation from section A, skim milk solution and Tween 80 separately at 121°C, 115 psi for 15 min.

7. Aseptically add sterilized skim milk preparation and Tween 80 to ingredients in section A

8. Prepare antibiotic solutions and filter sterilize.

9. Allow medium to cool to approx 45-50°C and add antibiotics

10. Mix gently to avoid air bubbles and pour plates (20 ml per 9.0 cm plate).

11. Allow plates to dry in Laminar Flow Bench or similar before use.

Note: Leave plates to dry in Laminar Flow Bench and use directly. Otherwise store prepared plates inverted in polythene bags at 4°C. Use within 3 weeks to ensure activity of antibiotics.

XCPl

Compound	Source	Cat #	gms/L
A			
Oxoid Special Peptone	Oxoid	L72	10
KBr	Fisher	P205-500	10
$CaCl_2$	BDH	27588 4T	0.25
Agar	Difco	014-01	15
Soluble Potato Starch	J.T.Baker	4006-01	10
Crystal Violet (1% aqueous)	Fisher	C-581	0.15ml
B			
Tween 80	Fisher	T164 500	10.0ml
Cephalexin	Sigma	C-4895	10mg
Fluorocil	Sigma	F-6627	3mg
Tobramycin	Sigma	T-4014	0.16mg
Nystatin	Duchefa	N 0138	40mg

Preparation

1. Weigh out all ingredients in section A into a suitable container

2. Add 1000 m1 of distilled water

3. Dissolve ingredients

4. Add crystal violet

5. Sterilize 121°C, 115 psi for 15 min.

6. Sterilize 10 ml Tween 80 separately at 121°C, 115 psi for 15 min.

7. Aseptically add Tween 80 to ingredients in section A

8. Prepare antibiotic solutions and filter sterilize and aseptically add to ingredients in section A

9. Allow medium to cool to approx 45-50°C and add antibiotics

10. Mix gently to avoid air bubbles and pour plates (20 ml per 9.0 cm plate).

11. Allow plates to dry in Laminar Flow Bench or similar before use.

Note: Leave plates to dry in Laminar Flow Bench and use directly. Otherwise store prepared plates inverted in polythene bags at 4°C. Use within 3 weeks to ensure activity of antibiotics.

Yeast Dextrose Chalk (YDC) agar medium

Compound	Source	Catalogue #	g/L
Bacto Agar	Difco	0140-01	15
Yeast Extract	Difco	0127-17-9	10
$CaCO_3$ (light powder)	J.T. Baker	1301-01	20
Glucose	Difco	0155-17-4	20
Distilled water			1000ml

Preparation

1. Weigh out all ingredients into a suitable oversize container (i.e. 250 ml of medium in a 500 ml bottle/flask) to allow swirling of medium just before pouring.

2. Add 1000 ml (or 500 ml) of distilled water.

3. Steam to dissolve.

4. Autoclave at 121°C, 115 psi for 15 min.

5. Allow medium to cool to 45-50°C.

6. Swirl to ensure even distribution of CaCO3 and avoid air bubbles and pour plates (22 ml per 9.0 cm plate).

7. Leave plates to dry in Flow Bench or similar before use.

Storage

Store prepared plates inverted in polythene bags.

Prepared plates can be stored for several months provided they do not dry out.

Part II
Preparation and composition of the previous Milk Tween (MT) media (1999)

MT

Compound	Source	Cat #	gms/L
A			
Proteose peptone #3	Difco	0122-17-4	10
$CaCl_2$	BDH	27588 4T	0.25
Tyrosine	Sigma	T-1145	0.5
Agar	Difco	0140-01	15
Distilled water			500
B			
Skim milk powder	Oxoid	L31	10
Distilled water			500
C			
Tween 80	Fisher	T164 500	10ml
D			
Cycloheximide	Duchefa	C-4698	200mg
Cephalexin	Sigma	C-4895	80mg
Vancomycin	Sigma	V-2002	10mg

Preparation

1. Weigh out all ingredients in section A into a suitable container

2. Add 500 ml of distilled water

3. Dissolve ingredients

4. Dissolve skim milk powder separately in 500 ml water

5. Separately Prepare 10 ml Tween 80

6. Sterilize, preparation from section A, skim milk solution and Tween 80 separately at 121°C, 115 psi for 15 min.

7. Aseptically add sterilized skim milk preparation and Tween 80 to ingredients in section A

8. Prepare antibiotic solutions and filter sterilize.

9. Allow medium to cool to approx 45-50°C and add antibiotics

10. Mix gently to avoid air bubbles and pour plates (20 ml per 9.0 cm plate).

11. Allow plates to dry in Laminar Flow Bench or similar before use.

Note: Leave plates to dry in Laminar Flow Bench and use directly. Otherwise store prepared plates inverted in polythene bags at 4°C. Use within 3 weeks to ensure activity of antibiotics.

Part III
Composition of Tween A Medium (modified) (TSM)

Compound	g/L
A	
Peptone	10
KBr	10
$CaCl_2$	0.25
Agar	15
Distilled water	1000ml
B	
Tween 80	10ml
C	
Cephalexin	35mg
Fluorouracil	12mg
Cyclohexamide	50mg (dissolved in 75% ethanol)

Autoclave A and B separately and allow to cool to 50^0C. Then add B and C to A.

Part IV
Composition of Modified Sucrose Peptone (MSP) medium

Compound	g/L
A	
Sucrose	15
Peptone	15
Agar	17
K_2HPO_4	3
NaH_2PO_4	1
$MgSO_4.\ 7H_2O$	0.3
Bromothymol blue (1.5% in 20% ethanol)	1ml
pH	7.2
B	
Cephalexin	80mg
Cychloheximide	200mg
Vancomycin	10mg

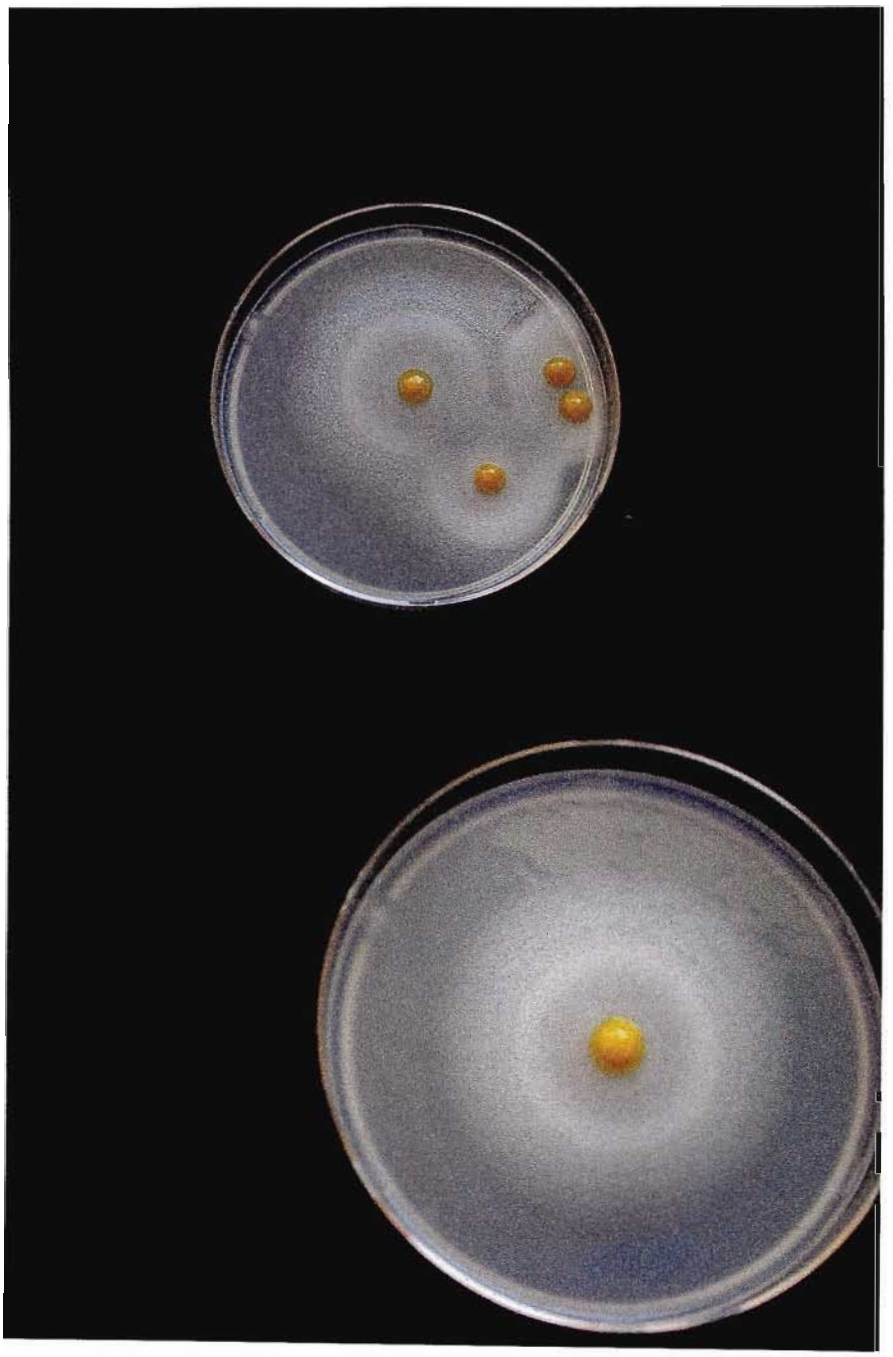
Autoclave A and allow to cool to 45^0C. Add B to A.

APPENDIX 2
Composition of Buffers (Reagents) used in ELISA

Buffer	Composition	g/L
Phosphate buffered saline PBS-T (Ph 7.4)	NaCl	8.0
	KH_2PO_4	0.2
	$Na_2HPO_4.H_2O$	2.9
	KCl	0.2
	NaN_3	0.2
	Tween 20	0.5ml
Coating Buffer (pH 9.6)	Na_2CO_3	1.59
	NaHCO3	2.93
	Na_3	0.2
Sample Extraction Buffer	PBS-T	
	40 Polyvinyl pyrrolidone	20
Substrate Buffer	Diethanolamine	97ml
	H_2O	800ml
	NaN_3	0.2
Substrate	Tablets	5mg per tablet
Enzyme	Alkaline Phosphatase	

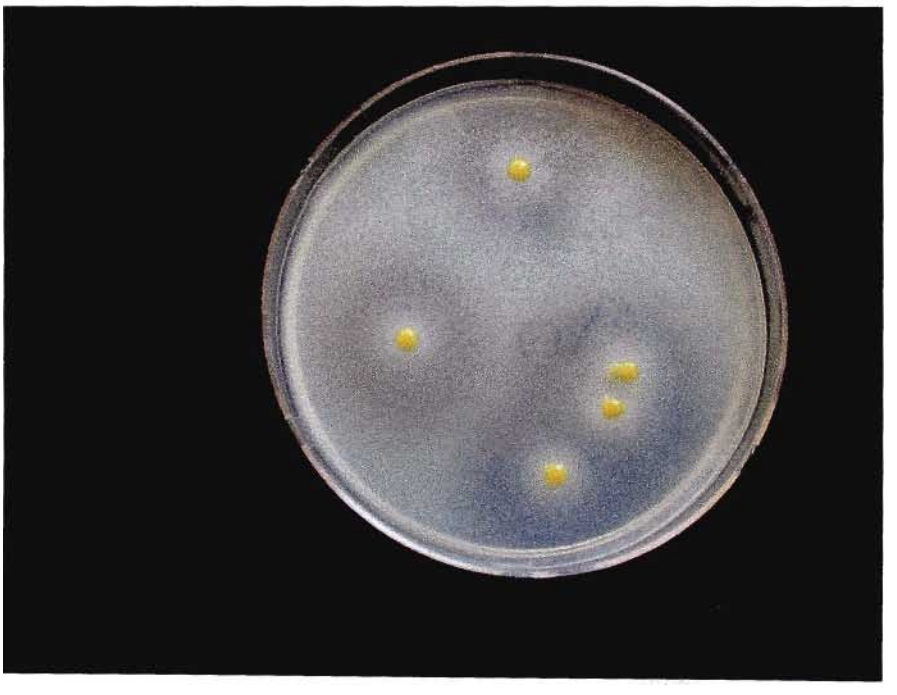
APPENDIX 3

Colonies of *Xanthomonas axonopodis* pv. *phaseoli* on XCP1, MT_{new}, MT and TSM

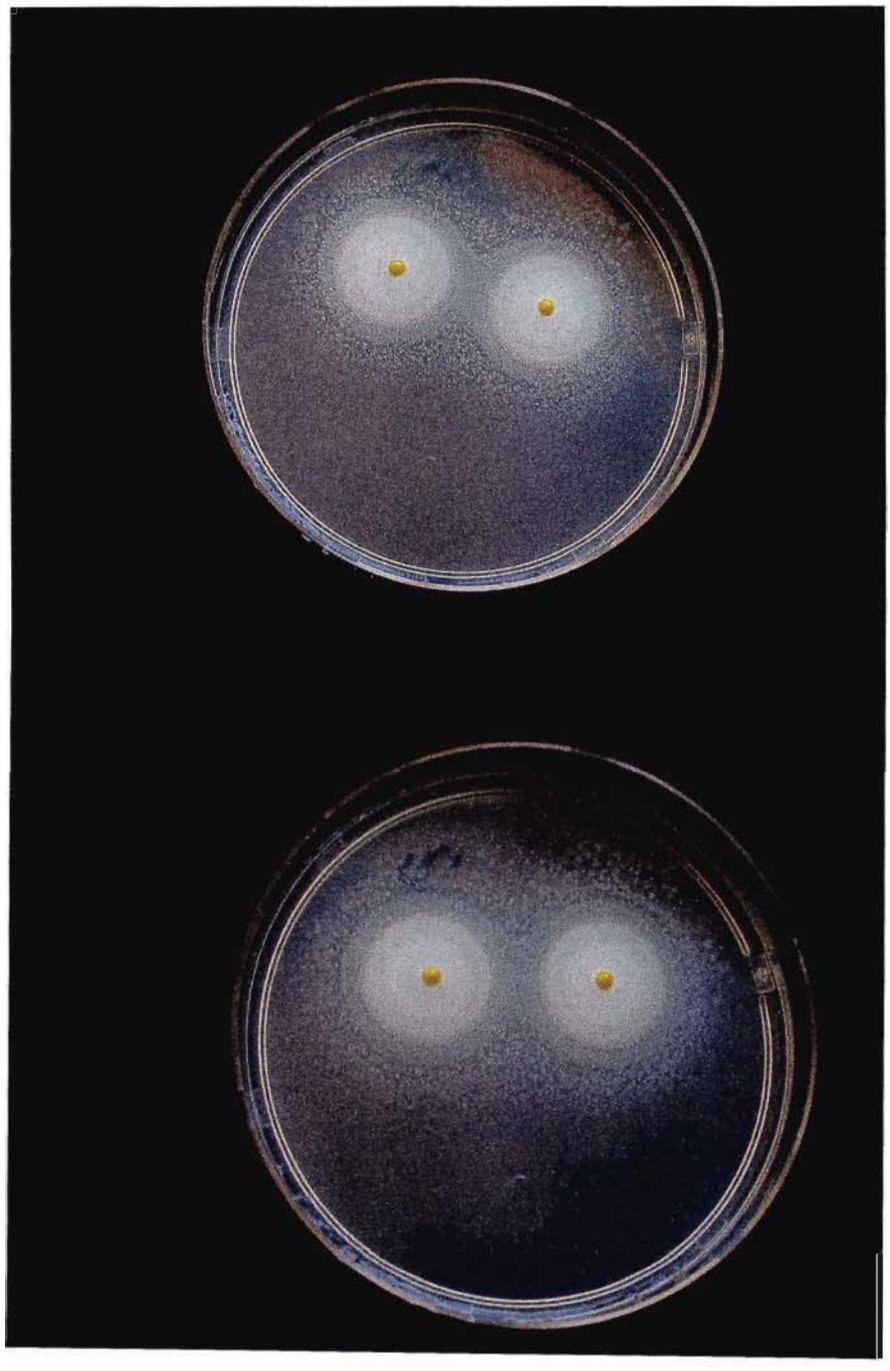


Xanthomonas axonopodis pv. *phaseoli* on XCP1

Xanthomonas axonopodis pv. *phaseoli* on MT_{new}



Xanthomonas axonopodis pv. *phaseoli* on MT



Xanthomonas axonopodis pv. *phaseoli* on TSM

APPENDIX 4

List of Raw Data

Raw data for detection of bacteria experiments

Number of *Pseudomonas syringae* pv. *phaseolicola* (Psp) colonies on the two media MT and MSP recorded from four experiments

Experiment	Rep	No. of Psp colonies in media		LSD	cv%
		MT	MSP		
I	1	150	132	17.27	8.6
	2	136	114		
	3	148	150		
	4	146	119		
	5	140	145		
II	1	103	114	22.62	13.6
	2	135	128		
	3	108	135		
	4	101	96		
	5	117	100		
III	1	5	20	5.63	27.3
	2	8	17		
	3	14	16		
	4	15	13		
	5	16	18		
IV	1	11	21	6.00	30.0
	2	16	16		
	3	12	11		
	4	15	7		
	5	16	12		

Number of *Xanthomonas axonopodis* pv. *phaseoli* (Xap) colonies on the two media MT and MSP recorded from four experiments

Experiment	Rep	No. of Psp colonies in media		LSD	cv%
		MT	MSP		
I	1	151	116	13.02	7.1
	2	148	85		
	3	144	100		
	4	154	105		
	5	158	96		
II	1	73	73	11.24	10.6
	2	77	71		
	3	86	51		
	4	73	70		
	5	84	70		
III	1	12	17	5.00	27.6
	2	23	13		
	3	13	9		
	4	18	9		
	5	21	10		
			9		
IV	1	24	12	5.02	24.9
	2	22	16		
	3	13	14		
	4	17	7		
	5	14	15		

Number of *Xanthomonas axonopodis* pv. *phaseoli* (Xap) colonies on four media (XCP1, MT, MT_{new} and TSM) plated from seven samples of seeds in the ISTA/ISHI Comparative Test conducted at Roodeplaat in 2003

Sample	Rep	No. of Xap colonies in media				LSD	cv%
		XCP1	MT_{new}	MT	TSM		
I	1	250	280	250	300	52.86	6.8
	2	250	300	300	300		
II	1	170	230	170	300	101.0	18.6
	2	130	162	104	300		
III	1	43	38	39	77	19.68	13.5
	2	62	37	41	83		
IV	1	72	104	104	127	35.86	13.7
	2	67	109	90	94		
V	1	74	41	31	87	36.61	26.0
	2	41	32	26	73		
VI	1	96	125	114	176	32.92	9.5
	2	95	93	114	186		
VII	1	77	52	23	77	23.92	16.5
	2	55	46	18	70		

Number of seed contaminant colonies on four media (XCP1, MT, MT_{new} and TSM) plated from six samples of seeds in the ISTA/ISHI Comparative Test conducted at Roodeplaat in 2003

Sample	Rep	No. of seed contaminant colonies in media				LSD	cv%
		XCP1	MT_{new}	MT	TSM		
I	1	40	111	82	1	11.06	7.0
	2	35	112	72	0		
II	1	88	71	47	76	28.1	15.1
	2	71	75	52	54		
III	1	11	50	50	75	8.44	6.5
	2	6	57	50	75		
IV	1	5	3	14	15	18.02	57.1
	2	2	5	14	33		
V	1	295	195	45	144	142.6	33.3
	2	152	220	47	138		
VI	1	55	16	8	16	4.28	6.7
	2	52	5	8	13		

Raw data for virus detection experiments

ELISA and symptom assessment results of seedlings grown from seeds harvested from bean common mosaic virus (BCMV: isolates BCMV-R and BCMV-U) inoculated plants

Seed source	Rep	No. of seedlings with +ve test result for		No. of dead seedlings with +ve ELISA
		ELISA	Symptom assessment	
BCMV-RE	1	1	4	1
	2	0	4	1
BCMV-RL	1	9	4	4
	2	7	6	4
BCMV-UE	1	6	4	1
	2	4	4	1
BCMV-UL	1	8	6	5
	2	8	6	5
LSD		2.94	1.96	2.77
cv%		19.7	14.9	36.4

Raw data for germination experiments

Percent of normal and abnormal seedlings, and dead seeds in a germination study for seeds harvested from *Xanthomonas axonopodis* pv. *phaseoli* (Xap), *Pseudomonas syringae* pv. *phaseolicola* (Psp), bean common mosaic virus (BCMV: isolates BCMV-R and BCMV-U) inoculated plants and uninoculated control (C) plants and electrolytic conductivity of seed soaks

Seed source	Rep	% of normal seedlings	% of abnormal seedlings	% ofdead seeds	Conductivity in µs/gm of seed
C	1	88	12	0	600
	2	88	12	0	620
	3	80	20	0	610
	4	88	12	0	590
Psp	1	56	44	0	530
	2	72	24	4	520
	3	60	36	4	570
	4	72	24	4	550
Xap	1	80	20	0	640
	2	60	40	0	630
	3	80	20	0	640
	4	84	16	0	640
BCMV-R	1	76	24	0	610
	2	80	20	0	690
	3	76	24	0	690
	4	88	12	0	660
BCMV-U	1	80	20	0	660
	2	84	16	0	640
	3	84	16	0	620
	4	84	16	0	620
LSD		10.38			33.53
cv%		8.8			3.6

Germination percent of seeds harvested from *Xanthomonas axonopodis* pv. *phaseoli* (Xap), *Pseudomonas syringae* pv. *phaseolicola* (Psp), bean common mosaic virus (BCMV: isolates BCMV-R and BCMV-U) inoculated plants and uninoculated control (C) plants, after aging

Seed source	Rep	Germination %
C	1	88
	2	80
	3	72
	4	80
Psp	1	36
	2	32
	3	28
	4	32
Xap	1	64
	2	52
	3	48
	4	44
BCMV-R	1	60
	2	70
	3	80
	4	68
BCMV-U	1	72
	2	64
	3	80
	4	64
LSD		10.98
cv%		11.9

Germination percent of undamaged seeds from infested lots of seeds harvested from *Pseudomonas syringae* pv. *phaseolicola* (Psp) and bean common mosaic virus (BCMV: isolates BCMV-R and BCMV-U) inoculated plants and uninoculated control (C) plants

Seed source	Rep	Germination %
C	1	84
	2	76
	3	88
	4	68
Psp	1	68
	2	68
	3	48
	4	48
BCMV-R	1	80
	2	72
	3	84
BCMV-U	1	68
	2	56
	3	72
	4	76
LSD		15.5
cv%		13.1