A COMPARISON OF SUSCEPTIBILITY TO FEEDING BY THE BLACK BEAN APHID (*APHIS FABAE*) BETWEEN TWO VARIETIES OF BEANS (*PHASEOLUS VULGARIS*) (GLORIA AND NUA 45) COMMONLY GROWN IN ZIMBABWE.

BY

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A dissertation submitted in partial fulfilment of the requirements for the Bachelor of Science Degree in Applied Biosciences and Biotechnology

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November 2017

APPROVAL FORM

This is to certify that the dissertation entitled "A comparison of susceptibility to feeding by the black bean aphid (*Aphis fabae*) between two varieties of beans (*Phaseolus vulgaris*) (Gloria and Nua 45) commonly grown in Zimbabwe.", submitted in partial fulfilment of the requirements for Bachelor of Science Honours Degree in Applied Biosciences and Biotechnology at Midlands State University, is a record of the original research carried out by MALVERN T. MUTSIYO R111557M under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

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ABSTRACT

Gloria and Nua 45 are two of the most commonly grown sugar bean varieties in Zimbabwe. Beans are a cheap alternative source of protein and have been on high demand due to the ever-rising meat prices. Gloria and Nua 45 are being affected by the black bean aphid (*Aphis fabae*) feeding which is the most problematic insect pest of beans. The aphid feeds on foliage of plants resulting in stunted growth and reduced yields. A comparison of susceptibility to feeding by *A. fabae* was done to determine which one of the two varieties is least affected by the aphid. Two glasshouses containing both varieties of beans were used and one of the glasshouses was not inoculated with the aphid and this served as the control. The plant heights, leaf areas and 100-seed weights of the two varieties were compared after a 90-day period. The experimental design was Randomised Block Design (RBD) with each block having three replicates in each glasshouse. It was difficult to create homogeneity due to confounding factors (temperature and exposure to sunlight) in the glasshouses hence this choice of experimental design. Data collected on plant height, leaf area and 100-seed weight were subjected to one-way analysis of variance (ANOVA) for RBD using SPSS version 21. Dunnett test was done to compare each variety to the control. Multiple comparisons for the effect of aphid feeding on plant height, leaf area and 100-seed weight was done using Tukey *post hoc* tests. Both varieties in the glasshouse that was inoculated with the aphid were susceptible to feeding. However, the plant heights, leaf areas and 100-seed weights varied significantly ($p<0.05$) between the two varieties with the least values of these parameters being recorded for Gloria. The one-way analysis of variance showed that the plant heights, leaf areas and 100-seed weights of plants in the control glasshouse did not vary significantly ($p>0.05$). Therefore, this study indicated that Nua 45 is the least affected variety between the two (Gloria and Nua 45). Therefore, this study recommends that in areas infested by the black bean aphid (*Aphis fabae*) Nua 45 should be cultivated as it is the more resistant variety.

ACKNOWLEDGEMENTS

It is my pleasure to acknowledge the depth l owe to many people who have assisted me in coming up with this project. I would like to express special gratitude to my academic supervisor, Mr Bare, for his constant support and mentorship. Mr Bare kindly undertook the formidable task of reading this whole dissertation and I am grateful to him for his frank and helpful comments. Special thanks are extended to Mrs R. J. Mbiringa (Associate Research Scientist: Entomologist) for assisting me in coming up with this project. Her input in terms of continued assistance throughout my project was greatly appreciated and helpful. Special thanks are also extended to Dr T. Muteveri for his mentorship on data analysis. I would also like to thank all research scientists and research associates at DR&SS including Mr. Rwafa, Mrs. Chihera, Mr. N. Mutema and Dr. G. Chikwenhere for their support and assistance throughout my project. To all my friends Mr. G.R. Gurupira, Mr. H. T. Takawira, Ms. B. Chipatiso and other scientists that are not mentioned I owe them my thanks for giving me the benefit of their expertise and making this project a success.

DEDICATION

This project is dedicated to my late parents. This project is also dedicated to my uncle Dr S. Kuhudzai and Mrs C. Kuhudzai who have sacrificed everything to give me the most and best education possible.

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CHAPTER 1: INTRODUCTION

1.1 Background

The common sugar beans (*Phaseolus vulgaris*) are of a very important economic status (FAO, 2006). Dry beans were grown on 27.7 million ha in 148 countries in 2004 and total production was 18.7 million metric tons (MT) with a market value of more US$10.7 billion (FAO, 2004). Sugar bean originated in Central South America and is one of the most important grain legume for human direct consumption in the world (Jones and Corlett, 1992). Developing countries produce 86 % of the worldwide production of beans. The per capita consumption is the highest in East Africa in countries such as Burundi, Rwanda and Uganda (Chester, 1969). However, the leading bean producer and consumer is Latin America, where beans are a traditional, significant food, especially in Brazil, Mexico, the Andean Zone, Central America, and the Caribbean (Schwartz, Brick, Nuland and Franc, 1996).

Beans are nutritionally rich because they are a good source of protein, folic acid, dietary fibre and complex carbohydrates. Sugar beans are also known to be one of the best non-meat sources of iron, providing 23% to 30% of daily recommended levels from a single serving (Pachico,1993). Beans are mostly consumed especially in developing countries because they are a cheap alternative source of protein and they are a means of keeping malnutrition at bay. Qualitatively, bean seed proteins provide essential amino acids such as lysine, but are poor in methionine, thus complementing cereals in this respect (Bressani, 1983; Gepts and Bliss,1985). Beans are also the third most important major source of calories (Pachico,1993). In addition, sugar beans play an important role as a source of minerals, especially iron and zinc (Chester, 1969), for which it also complements cereals. Genetic variation for seed content of these minerals has been demonstrated (Lynch, Lauchli and Epstein, 1991).

Constraints to common bean production are poor production practices, pests and diseases, low soil fertility, adverse weather conditions, weed infestations and plant populations (Wortman and Allen, 1994; Gridley and Danial, 1995). According to the Ministry of Agriculture, Mechanization and Irrigation, the Department of Research and Specialist Services Gloria and Nua 45 are the two varieties of sugar beans that are commonly grown in Zimbabwe and the most problematic pest of these two varieties is the black bean aphid (*Aphis fabae*). Wilkinson and Douglas (2003) state that *Aphis fabae* causes economic damage mainly due to direct feeding. Aphids tap into the phloem, and to a lesser extent the xylem, with their stylets and ingest large quantities of soluble nutrients.

Beans suffer damage to flowers and pods which may fail to develop properly. Early-sown crops may avoid significant damage if they have already flowered before the number of aphids builds up in the spring (Berim,2009). For the aphids to obtain enough protein they need to suck large volumes of sap. They secret excess sugary fluid, honeydew, which adheres to plants where it promotes growth of sooty moulds. These reduce the surface area of the plant available for photosynthesis causing stunted growth, reduced yields and may reduce the value of the crop (Banks and Macaulay,1967). These aphids are also vectors of about thirty plant viruses, mostly of the non-persistent variety (Blane *et al*.,1990). The aphids may not be the original source of infection but are instrumental in spreading the virus through the crop (Rothamsted Research, 2003). Various chemical treatments are available to kill the aphids and organic growers can use a solution of soft soap (Godfrey and Trumble, 2009).

While substantial bean improvement work has been done by breeding improved cultivars, response to soil fertility and disease control (Makini and Danial, 1995; Tyagi *et al*., 1996; Maiuki, 1998) the susceptibility to feeding by the black bean aphid between Gloria and Nua 45 varieties has not been given due attention. No study has been carried out to determine which one of the two varieties of beans is least affected by *A. fabae*. Any advances in scientific research that benefit bean yields, particularly in developing countries, help to feed the hungry and give hope for the future. Identifying and minimizing yield limiting factors is an ongoing concern for many bean improvement programs. In addition, given the prevalence of beans in these diets, modifying the nutrient content in general to make it a more balanced and nutritious food source is also receiving emphasis. Hence this study is going to bridge that gap.

1.2 Justification

There has been an increased demand for beans due to the economic hardships that we are currently going through. The ever- rising prices of meat, which is also a source of protein has led to reliance on beans for subsistence (Katungi *et al.*, 2017). Institutions such as universities, boarding schools, prisons and hospitals rely on beans for their diets. Beans is a cheap alternative source of protein and a crop of economic importance. Other than proteins beans also contain minerals and fibre. Beans contain iron, about 95mg per kg and zinc, about 38 mg per kg (Norman, Pearson and Searle, 1995). This makes beans a key player in the reduction of anaemia in pregnant women and also helpful in individuals living with HIV/AIDS (Kaplan, 2008).

Knowing which variety between Gloria and Nua 45 is affected less by *A. fabae* will go a long way in helping farmers make a better decision when buying seed. Farmers will invest less in pesticide procurement, some of which are harmful to the environment. For instance, over 98% of sprayed pesticides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields. The knowledge from this study will also help in boosting the farmers' yields. Higher yields will help cater for the unfulfilled demand of the legume crop. The United Nations declared 2016 The International Year of Pulses to celebrate the growing importance of beans.

Gloria and Nua 45 are affected by the black bean aphid and this has been a challenge to farmers. It is unknown which variety between the two is least affected. The aphid populations cause a significant loss in the yield by decreasing flower and pod production. Yield loss in beans is as a result of fewer pods per plant and fewer and smaller seeds per pod. Seed viability and value is also reduced due to aphid feeding (Cammell and Way, 1983).

Farmers are investing too much capital in buying pesticides some of which are harmful to the environment. The pesticides DDT, methyl parathion and especially pentachlorophenol have been shown to interfere with legume-rhizobium chemical signalling. Reduction of this symbiotic chemical signalling results in reduced nitrogen fixation and thus reduced crop yields. This study helped to determine which one of the two varieties (Gloria and Nua 45) is less affected by the black bean aphid (*Aphis fabae)* hence reducing the environmental footprint of pesticides.

1.3 Objectives

1.3.1 Main objective:

- to compare the effect of aphid feeding between Gloria and Nua 45.

1.3.2 Specific objectives:

- to compare the effect of aphid feeding on the plant height of Gloria and Nua 45 over a 90-day period,
- to compare the effect of aphid feeding on the leaf area of Gloria and Nua 45 ,over a 90-day period, and
- to compare the effect of aphid feeding on the yield of Gloria and Nua 45 over a 90-day period.

CHAPTER TWO: LITERATURE REVIEW

2.1 History of beans (*Phaseolus vulgaris*) cultivation

Beans have been known to be cultivated since long ago (Kaplan, 2008). Seeds that had the size of a small fingernail were initially gathered in their wild state in Afghanistan and the Himalayan foothills (Kaplan, 2008). Cubero (1974) postulated a Near Eastern centre of origin, with four radii to Europe along the North African coast to Spain, along the Nile to Ethiopia, and from Mesopotamia to India (Hawtin and Hebblethpiait, 1983). Secondary centres of diversity are postulated in Afghanistan and Ethiopia.

Chester (1969) reported that beans were grown in Thailand as early as the seventh millennium (B.C). It is believed that the dead deposited them in ancient Egypt. Large seeded broad beans then appeared in the Aegean and transalpine Europe during the second millennium (B.C) (Daniel and Maria, 2000). However, Hajjar and Hodgkin (2007) reported the origin to be Central Asia. The Chinese used them for food almost 5,000 years ago, and they were cultivated by the Egyptians 3,000 years ago, by the Hebrews in biblical times, and a little later by the Greeks and Romans (Mihailovic *et al*., 2005; Singh and Bhatt, 2012). Probably, the Europeans introduced it as a garden crop into India during the Sultanic period (1206–1555), during which its cultivation has been mentioned (Naqvi, 1984; Akbar, 2000).

The oldest domesticated beans were found in Guitarrero cave, which is an archaeological site in Peru in the Americas. These beans dated to around the second millennium (B.C) (Chazan, 2000). Even back then beans were an important source of protein as they still are this modern age. The United States alone is responsible for the cultivation of more than 4,000 cultivars of beans. However, cultivars of beans that are commonly eaten fresh were first discovered in what is believed to be the Bahamas by a European by the name of Christopher Columbus during his exploration. Some tribes that dwelled on the East Coast of what is now known as the United States would grow maize, beans and squash intermingled them together in a system that had originated from Mexico (Everett, 2009).

2.2 Black bean aphid (*Aphis fabae*)

The black bean aphid, *A. fabae*, is widespread in temperate regions, where it is an important economic pest of sugar beans (*Phaseolus vulgaris*). Since the 1960s, *A. fabae* has become an important pest of *Phaseolus vulgaris* in East Africa (Eastop, 1977). The aphid causes great economic damage through direct feeding and is also responsible for indirect damage where it serves as a vector of plant viruses. It is of major economic importance because it causes direct feeding damage on beans, and feeding and virus transmission on *Beta vulgaris*. However, *A. fabae* is of relatively minor importance as a virus vector on other crops.

Dense aggregations of aphids on actively growing plants results in severe yield losses. Aphids tap their stylets deep into the phloem, and to a lesser extent the xylem, and ingest large quantities of soluble nutrients (Wilkinson and Douglas, 2003). Winged aphids may ingest more from the xylem to counter dehydration during flight (Powell and Hardie, 2002). Their saliva contains chemicals that may also disrupt plant physiology. Large infestations of aphids stunt plant growth, reduce seed formation, and may eventually cause premature plant death.

A. fabae has been known to cause serious losses in beans for a very long time (Curtis, 1960; Davidson, 1921; Cammell, 1981). Similar studies have shown that the aphids prefer the growing parts on *Vicia faba* (field bean, broad bean, faba bean). If young plants are severely attacked by dense infestations they may become stunted and may die early. Since these aphids suck on the sap which contains the plant nutrients, infestation slows the rate of stem elongation, leaf growth and root system development. An *A. fabae* nymph can ingest 3.5-4.5

mg of sap during its 7-day development period, while each adult consumes about 30 mg during its lifetime (Banks and Macaulay, 1964).

Aphid aggregations become less pronounced as the plant grows and the colonies become distributed around the plant. Populations on older plants, before or during flowering, can still cause significant crop losses, however, by decreasing flower and pod production. Yield loss in beans is primarily due to fewer pods per plant, and fewer and smaller seeds per pod. Damage also reduces seed viability and food value (Cammell and Way, 1983).

A 3-year study that was conducted in the former East Germany in the late 1970s estimated the yield losses caused by *A. fabae* in pot and field experiments. Yields were calculated in terms of the weight of 1000 seeds. The yields of uninfested beans were higher than that of the infested beans. Uninfested beans were around 615 g, while the lowest yields in plots heavily attacked by *A. fabae* at the time of flowering were around 380 g. It was observed that the levels of injuriousness caused by the *A. fabae* are dependent on the time and intensity of colonization. If plants are infested before flower buds form usually the plants totally perish before pods form. When aphid attack coincided with the onset of flowering, yields declined by 60-65% in pot experiments, compared with uninfested controls, while yields declined by around 50% in plot experiments. Later attacks, at the time of pod setting, caused yields to decline by 6% at most in equivalent experiments (Hinz and Daebeler, 1981).

In similar studies that were carried out in Britain, the beans that were sown in March were attractive to primary colonists, while crops sown in late April were susceptible to secondary colonists migrating from other bean crops. However, late autumn-sown or 'winter' beans were rarely damaged by *A. fabae* as their critical growth period occurs at a time when aphids are not present in the crop (Cammell and Way, 1983).

In experiments carried out in France, *A. fabae* and the pea aphid (*Acyrthosiphon pisum*) reduced yields (quantified as weight of 1000 seeds and number of seeds per pod) of *V. faba* by up to 30%, dependent on the physical condition of the plants (Bouchery, 1977). Higher yield losses due to *A. fabae* on *V. faba* were reported in a field study in Iraq, using artificial infestations. Pod number, pod weight and dry weight of seeds were negatively correlated with aphid numbers, the reductions in these parameters reached 70, 76 and 64%, respectively (Mohammad and Abdulla, 1988).

In a field study conducted in South America the length of time that *A. fabae* is present on *V. faba* has been correlated with yield loss. Early infestations (before the flowering period) resulted in considerable damage, dependent on the population levels attained in the aphid colonies. Early stage high infestations decreased yields (total weight of seeds) by up to 42% compared with controls. Infestation at the beginning of the flowering period has little effect on flower formation and development, although high populations at this stage may have an adverse effect on pod formation. Any infestations later than this stage had no effect on yield (Salazar, 1984).

2.3 Importance of beans (*Phaseolus vulgaris*)

2.3.1 Nutritional value

Sugar beans are characterized as a near perfect food by nutritionists because they contain a high protein content (20%-24%), complex carbohydrates and other much needed dietary necessities (Bliss,1980; Lincoln,1987). Sugar beans also provide the recommended daily levels of certain trace minerals that include iron (25%-30%), magnesium and copper (25%) and potassium and zinc (15%) (Peters,1993). Dry beans (22% protein content) have a wide range of dishes which include simply boiling them in water and even more sophisticated preparations which provide us with products such as salads, soups, baked beans, pastes, chips and creams (Hosfield, 2000).

Dry leaves, threshed pods, and stalks are fed to animals and used as fuel for cooking, especially in Africa and Asia. In Peru and Bolivia, where high altitudes prolong cooking times and fuel costs, the ancient tradition of toasting grains comparable to corn and peanuts may be the reason why popping or "toasted" beans have been developed. They are cooked similarly to popcorn. Dry beans are mostly eaten whole in cooked recipes. Some manufactured products use bean flour. Roasted beans can be pin-milled to produce whole flour or cracked by corrugated rollers for easy removal of hulls by air aspiration. Hulls may be ground as high fibre (40 percentage) flour to desired particle size (Sperling,1996).

2.3.2 Medicinal value

Beans have demonstrated impressive health benefits that include lowering cholesterol levels, improving diabetics' blood glucose, reducing risk of many cancers, lowering blood pressure, regulating functions of the colon, preventing and curing constipation, preventing piles and other bowel problems.

A lesser-known benefit of beans, though, is their high levels of isoflavones, compounds that are similar in structure to estrogen produced by the body (which is why they are also called phytoestrogens). These isoflavones may ease the symptoms of menopause, prevent some form of cancer, reduce the risk of heart disease and improve bone and prostate health, among other benefits.

2.3.3 Income generation

Despite the production being low the price of beans has been trending upwards on the local market. Current production levels in Zimbabwe are relatively low compared to other countries like Malawi and Tanzania. The margins of growing sugar beans are larger than that of growing any other food crop for example maize (World Vision, 2003). The key to getting profitable with bean farming is choosing a variety that does not have high input costs such as costly seed and is not highly affected by pests such as the black bean aphid (*Aphis fabae*). Farmers would prefer to invest less capital in pesticides procurement some of which are also harmful to the environment.

2.3.4 Improvement of soil health

Beans are nitrogen fixing and can improve the fertility of soil. Good soils help to grow healthier, more resilient crops and improve crop yields. Beans use less water to grow, they are water-efficient.

2.4 Current status of sugar bean production in Zimbabwe

Currently in Zimbabwe the national yield is still significantly low such that the domestic market is failing to meet local demands. These local demands are attributed to the high consumption of beans in prisons, hospitals, boarding schools, universities and restaurants. The Agricultural Development and Market Corporation of Malawi sells about 100 MT to 150 MT of their beans to their domestic market and 80-85% of their produce is exported to Zimbabwe and the rest to South Africa (World Vision,2003).

However, it is not known which variety is least susceptible to aphid feeding between the two sugar bean varieties (Gloria and Nua 45). This study will go a long way on educating the farmers about the variety that is least affected by the black bean aphid (*Aphis fabae*). This will help farmers have better yields especially in areas where there is a high occurrence of the aphid.This study will also help farmers to invest less in pesticide procurement some of which are harmful to our environment.

2.5 Factors affecting sugar bean production

2.5.1 Biotic factors

Pests

Besides the black bean aphid which was used in this study a number of other pests attack beans. These include the bean stem maggot, bean stem maggot, bean bug, common cutworm, bean flower thrips, bean weevil and the bean gail weevil.

Diseases

A wide range of diseases are known to attack beans and these include Fusarium species, Halo blight, Powdery mildew, Common blight, Bean Common Mosaic Virus(BCMV), Angular leaf spot, Bacterial brown spot, Pythium species and Ascochyta. All the listed diseases cause significant damage in beans which affects the crop and may cause reduction in yields (Norman *et al*,1995).

Weeds

Weeds reduce crop yields. Every living plant requires a certain amount of space for the circulation of air, moisture and sunlight. When plants are crowded and this much-needed space is occupied by weeds then they cannot grow and produce well. The reproductive behaviour and the growth rate of the plant is affected and returns from the plant will be correspondingly less (Vital, 2001).

2.5.2 Abiotic factors

Use of uncertified seed

The production of beans is inhibited by the use of uncertified and retained seed. Some farmers tend to use seed of the beans they harvested the past season and stored under traditional conditions where they can easily get infested by post-harvest bean weevils resulting in poor emergence (Dube and Nyoka,1993).

3.0 MATERIALS AND METHODS

3.1 Study site

This study was carried out at the Department of Research and Specialist Services in Harare which is under the Ministry of Agriculture, Irrigation and Mechanization. The experiments were carried out in the glasshouses, Entomology and Pathology laboratories.

3.2 Sources of materials

The Gloria and Nua 45 bean seeds used in this study were provided by the Plant Protection department after having been checked for their viability and after being confirmed to be disease-free in the Pathology laboratory. The seeds were planted into their respective pots containing soil which had been collected from the DRSS fields. The aphids (black bean aphid) used in this research were reared on a potted bean plant after being collected from infested fields in Zaka. To avoid injuring the aphids, an instrument called the aphid gun was used. Two glasshouses were used to carry out the experiment. The first glasshouse contained plants exposed to the treatment (presence of the aphid). The second glasshouse was the control glasshouse and this had plants growing under normal conditions free from the influence of the treatment.

3.3 Experimental design

Randomized block design (RBD) was used during this experiment. The blocks were randomly assigned to block out the effect of temperature and exposure to sunlight (Figure 1.1 and 1.2). These two were found to be confounding factors in the glasshouses and it was difficult to create homogeneity among our experimental units hence this choice of experimental design. The first block contained 10 plants of Gloria and the second block contained 10 plants of Nua 45. Each of the two blocks had three replicates thus giving a total of four blocks containing each bean variety. Each glasshouse thus had a total of 8 randomly assigned blocks. The other glasshouse acted as the control and *Aphis fabae* was not inoculated in this one.

Nua 4	**Nua 2**
Nua 3	**Glo 1**
Glo 4	**Glo 3**
Nua 1	**Glo 2**

Figure 1.1: Arrangement of blocks in Glasshouse 1 where the black bean aphid (*Aphis fabae*) was inoculated.

Nua 3	**Nua 4**
Glo 2	**Glo 1**
Nua 1	**Glo 3**
Nua 2	**Glo 4**

Figure 1.2: Arrangement of blocks in Glasshouse 2 which served as the control.

Key

Glo- Gloria   Nua- Nua 45

3.4 Rearing of aphids

A single mother of apterous adult aphid collected from infested fields in Zaka was used to initiate black bean aphid culture on the bean plant. Prior to the experiment, the black bean aphid culture had already been running for 4 weeks. New bean seedlings were provided continuously to replace old ones for the maintenance and continuous growth of the black bean aphid cultures.

3.5 Preparation of sample plants

This study was carried out in two separate glasshouses and each glasshouse had a total of 80 plants, that is, 40 for each variety under study. The seeds were planted in pots that were filled with fine loam soil at a density of three seeds per pot and were placed 2.5 to 5.0 cm below the soil surface. Seeds were planted in plastic pots (20cm diameter) and seven days after emergence, seedlings were thinned to a single plant for each pot to avoid plants competing for nutrients.

3.5.1 Pathological seed analysis

The seeds were tested to see if they were free from disease and healthy using the blotter test method (Zad,1987). Prior to the blotter test, seeds were sterilised in 1% sodium hypochlorite for 5 minutes to free or reduce superficial microorganisms (Sharma *et al*., 1997). The seeds were then placed on layers of water soaked paper in petri dishes and incubated for 7 days at 20℃.

3.5.2 Soil sampling

Soil used in this study was collected from the DRSS fields using a spade at a depth of 0-50cm. The soil was air dried under shade and ground with a wooden roller and then passed through a 2-mm sieve. The pH of the soil used in this study was tested using a pH test kit. The soil in the pots had a depth of 90 cm. All the soil used in this research was made as free as possible of clumps of earth or sod by manually destroying lumps of soil using hands.

3.5.3 Watering and fertilisation

The plants were drenched with water every two days to ensure that they received adequate water supply for photosynthesis and to maintain soil moisture. Shortly after emergence the plants were fertilised with a 5:10:10 blend of synthetic fertilizer with four granules per plant. Fertiliser was applied to all the plants in both glasshouses including the one which served as the control.

3.5.4 Weed control

Seeds were planted in soils that were already free from weeds as beans are slow growing plants and do not easily overshadow weeds. Weeds that emerged during the experiment were removed manually by pulling them out using hands.

3.6 Determination of the effect of the Black bean aphid (*Aphis fabae*) on the plant height, leaf area and yield of Gloria and Nua 45.

3.6.1 Inoculation of sample plants with the Black bean aphid (*Aphis fabae*)

Since young plants are very susceptible to aphid feeding, the *Aphis fabae* was inoculated 14 days after plant emergence. Ten 3 to 5 days old nymphs (apterous) were introduced on each plant and covered with clear PVC tubes (90 cm high by 30 cm in diameter) and ventilated using muslin cloth. Aphids were only inoculated in the first glasshouse whilst no aphids were inoculated at all in the other glasshouse which served as the control. To avoid injuring the aphids an aphid gun was used to transfer the aphids from the rearing unit onto the sample plants.

3.6.2 Measuring of plant height

At day 90 (maturity), 6 randomly selected plants from each block had their height measured in cm. Sampled plants were measured from the ground level to the tip of the plant using a ruler and a tape measure.

3.6.3 Determination of leaf area

The areas of 5 randomly selected leaves on each of the 6 randomly sampled plants in each block were assessed at maturity at day 90 in the glasshouse. Leaf area measurements were performed early in the morning, when there was more diffuse and less direct solar radiation. The leaf areas were measured using leaf area metre (L.I 3100, Li-Cor.inc, Lincoln, NE). Dead and senescent leaves were disregarded in determinations of leaf area.

3.6.4 Measuring of yield

At maturity, the 100-seed weight was recorded for each of the 6 randomly selected plant from each block.

3.7 Data analysis

Analysis of variance was conducted using one-way ANOVA for RBD in SPSS. There was one predictor variable (factor) that is, bean variety with two factor levels (Gloria and Nua 45) and three response variables that is leaf area, plant height and yield (100-seed weight). A one -way ANOVA was conducted to compare the susceptibility to feeding by the black bean aphid (*Aphis fabae*) between two varieties of beans commonly grown in Zimbabwe, that is, Gloria and Nua 45. Dunnett test was used to compare the susceptibility to feeding by the black bean aphid (*Aphis fabae*) between two varieties of beans (Gloria and Nua 45) to the control.

CHAPTER FOUR: RESULTS

4.1 Effect of *A. fabae* on the plant heights, leaf areas and 100-seed weight (yield) of Gloria and Nua 45 plants

Aphis fabae feeding had an effect on the plant heights, leaf areas and 100-seed weight of Gloria and Nua 45 ($p<0.05$). Generally, Gloria was more susceptible to feeding by the black bean aphid (*Aphis fabae*) more than Nua 45 (Table 1) ($p<0.05$). The sample plants in the glasshouse where the aphid was inoculated had reduced plant heights, leaf areas and 100-seed weight (yield) compared to control plants where the aphids were not inoculated ($p<0.05$). Table 1 shows the means for the plant height, leaf area and 100-seed weight of the 6 plants that were randomly selected from each block at maturity.

Table 1: Effect of *A. fabae* on the mean plant heights, leaf areas and 100-seed weight (yield) of Gloria and Nua 45.

Block	Plant heights	Leaf areas	100-seed weight
Glo 1	26.27	35.16	35.75
Glo 2	26.70	39.30	38.10
Glo 3	27.37	29.80	34.83
Glo 4	28.01	36.47	36.31
Nua 1	41.71	43.05	46.23
Nua 2	44.32	46.46	45.72
Nua 3	43.76	48.94	44.70
Nua 4	45.22	45.26	43.95

The mean plant height ranged from 26.27 cm to 45.22 cm for both varieties and the mean leaf areas ranged from 29.80 cm^2 to 48.94 cm^2. The 100-seed weights ranged from 34.83g to 46.23g (Table 1).

Table 2: Mean plant heights, leaf areas and 100-seed weights of the control plants (where the aphid was not inoculated)

Block	Plant heights	Leaf areas	100-seed weight
Glo 1	61.24	58.06	58.44
Glo 2	62.48	54.97	62.21
Glo 3	63.75	56.62	61.21
Glo 4	61.30	55.81	63.37
Nua 1	62.24	59.01	61.96
Nua 2	61.93	57.85	60.39
Nua 3	64.24	57.72	62.68
Nua 4	62.70	57.25	62.55

The mean plant heights in the control glasshouse ranged from 61.24 cm to 64.24cm for both varieties and the mean leaf area ranged from 54.97cm^2 to 59.01cm^2. The mean 100-seed weight ranged from 58.44g to 62.68g. The mean plant height, leaf area and 100-seed weight were significantly different ($p<0.05$) from those of plants in the glasshouse where the aphid was inoculated.

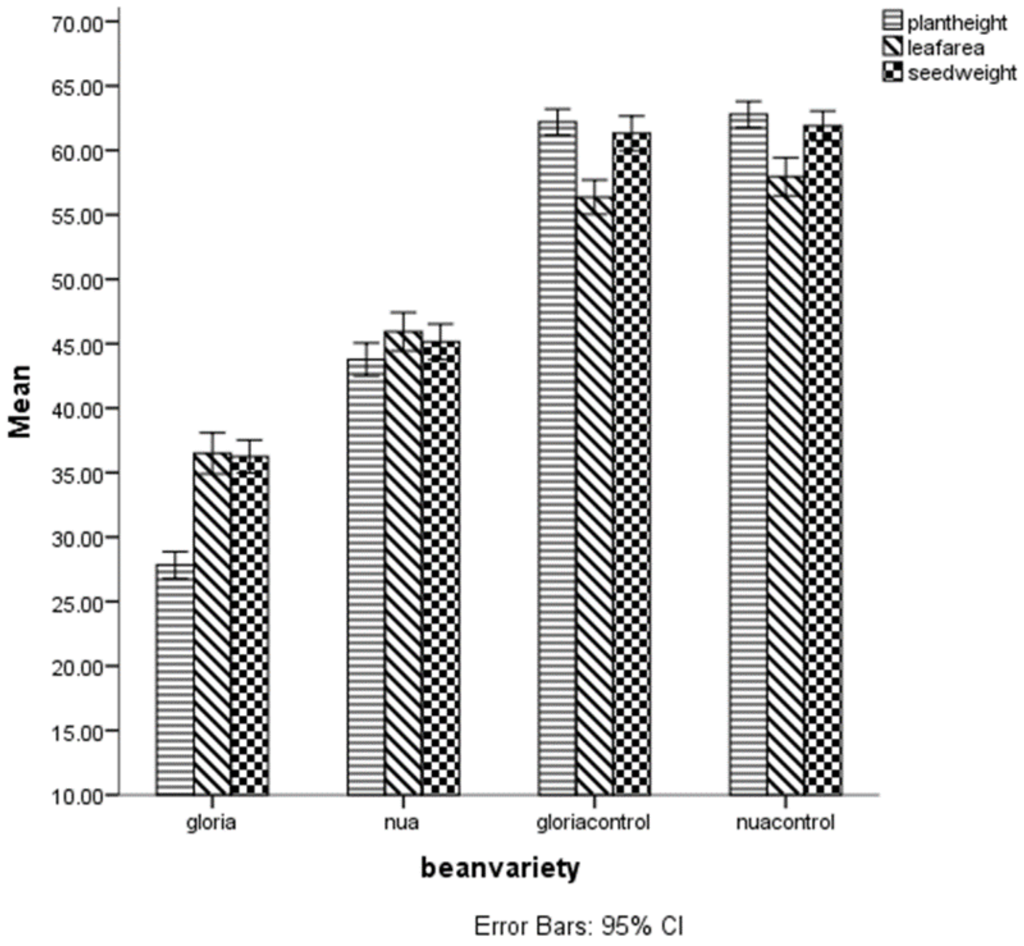

Figure 2: Effect of *Aphis fabae* feeding on Gloria and Nua 45 compared to the control

Figure 2 is showing the mean plant height, leaf area and the 100-seed weights of the sample plants that were in the glasshouse where the *Aphis fabae* was inoculated compared to those of the plants that were in the control glasshouse where the aphid was not inoculated.

The results in Table 1 and Figure 2 show that the least mean plant height (26.27cm) in the inoculated glasshouse was recorded for Gloria. The least mean leaf area (35.16cm^2) was recorded for Gloria and the least mean 100-seed weight (34.83g) was also recorded for Gloria.

The results in Table 2 and Figure 2 show that the mean plant height ranged from 61.24 cm to 64.24 cm for both varieties. The mean leaf area ranged from 55.81 cm^2 to 59.01 cm^2 and the mean 100-seed weight for both varieties ranged from 58.44g to 63.37g.

Tukey post hoc test revealed that there was a significant difference in the plant heights, leaf areas and 100-seed weights between the Gloria and Nua 45 plants that were in the glasshouse which was inoculated with the aphid (ANOVA: $p<0.05$), confirming differences shown in Fig 2.

Dunnett post hoc pairwise comparisons showed no significant differences between the plant heights, leaf areas and 100-seed weight of the Gloria control plants and Nua 45 control plants (ANOVA: $p>0.05$). However, all the sample plants in the inoculated glasshouse were significantly different (ANOVA: $p<0.05$) from the control.

CHAPTER FIVE: DISCUSSION

5.1 Effect of *Aphis fabae* feeding on the plant heights of Gloria and Nua 45

All the plants of the two varieties used in this study were susceptible to feeding by the black bean aphid (*Aphis fabae*). The plant heights of Gloria and Nua 45 were reduced but up to varying degrees. This was because the aphids feed by sucking plant juices and remove sap which creates a lack of vigour in the plant and reduces plant height. Gloria was the most susceptible because it had the most reduced plant heights. This is because the aphid preferred feeding on the Gloria plants more than the Nua 45 plants. This could have been due to different plant quality between the two varieties. Aphids tend to change feeding locations more often when feeding on poor food sources compared with higher quality food sources (Dixon, 1998). Previous work has shown that on preferred host plants, aphids have short walking times and prolonged probing periods, in this case this could have been the Gloria plants whereas the opposite behaviour is observed on non-preferred host plants, for instance, Nua 45 plants in this study (Traicevski and Ward, 2002).

5.2 Effect of *Aphis fabae* feeding on the leaf areas of Gloria and Nua 45

Gloria also had the most reduced leaf areas compared to Nua 45 but both varieties were susceptible to aphid feeding and had their leaf areas reduced. However, Nua 45 seemed more resistant to aphid infestation better than Gloria. Reduction in leaf area was because of the toxins found in the saliva that is injected into plants by the black bean aphid, which causes leaves to curl and reduce their surface area. Aphids produce two different types of saliva (Miles, 1999). The first type is dense and proteinaceous, and, jellifying around the stylets (stylet sheaths), it constitutes an intercellular path towards the phloem for the piercing stylets, isolating plant tissues from the mouthparts, thus preventing plant reaction at the site of feeding (Felton and Eichenseer, 1999). When the stylets have reached the phloem flow, aphids start to produce the second type of saliva, referred to as ‘watery’, that is injected directly into the vascular system of the plant (Douglas, 2006).

The aphids also secrete excess sugary fluid called honeydew which adheres to plants where it promotes growth of sooty moulds. This also reduces the surface area of the plant available for photosynthesis and may reduce the value of the crop. This is the reason why the plants of both varieties in the control had much increased leaf areas.

5.3 Effect of *Aphis fabae* feeding on the 100-seed weight of Gloria and Nua 45

Gloria had much reduced 100-seed weights than Nua 45 and this is because Nua 45 had a better compensatory effect where it managed to compensate for the loss that was being caused by aphid feeding. The aphids were more localised in Nua 45 plants than in Gloria and this allowed compensation to occur. Aphid feeding causes damage to flowers and pods which may not develop properly. Flower and pod formation may abort due to the action of the toxic saliva injected by the aphid to improve the flow of sap. Present findings were close to those of studies carried out in France, where *A. fabae* reduced yields (quantified as weight of 1000 seeds and number of seeds per pod) of *V. faba* (another variety *of Phaseolus vulgaris*) by up to 30% (Bouchery, 1977). Higher yield losses due to *A. fabae* on *V. faba* were also reported in a field study in Iraq, using artificial infestations. Pod number, pod weight and dry weight of seeds (yield) were negatively correlated with aphid numbers, the reductions in these parameters reached 70, 76 and 64%, respectively (Mohammad and Abdulla, 1988).

A similar study by Salazar (1984) showed that the length of time that *A. fabae* is present on *V. faba* has been correlated with yield loss in a field study in South America. Early infestations (before the flowering period) resulted in considerable damage, dependent on the population levels attained in the aphid colonies. High infestations at this early stage decreased yields (total weight of seeds) by up to 42% compared with controls. Infestation at the beginning of the flowering period has little effect on flower formation and development, although high populations at this stage may have an adverse effect on pod formation. Any infestations later than this stage had no effect on yield.

5.4 Conclusion

Gloria was more susceptible to aphid feeding, with Nua 45 showing to be more resistant over a 90-day period. Trends from the results revealed that Gloria plants that were in the glasshouse inoculated with *A.fabae* had the most reduced plant heights, leaf areas and 100-seed weights. However, both varieties were susceptible to feeding *by A.fabae* although in varying degrees because the control plants where the aphis was not inoculated had much increased plant heights, leaf areas and 100-seed weights. Hence between the two varieties that were under study, Nua 45 is the least affected by the *A.fabae*.

5.5 Recommendations

This study recommends that in areas where there is occurrence of *A.fabae* farmers cultivate the Nua 45 variety as it is more resistant to the aphid feeding. Moreover, farmers will have higher yields and invest less in pesticides procurement some of which are harmful to the environment and may cause pest outbreaks. Damage and economic loss caused by *A. fabae* can also be avoided through the use of agricultural practices such as intercropping the bean varieties with maize.

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APPENDICES

Appendix 1: Pathological seed analysis using Blotter test

1. Sterilise seeds in 1% sodium hypochlorite for 5 minutes.

2. Soak blotter paper in distilled water then place it in a petri dish in the laminar flow.

3. Place 5 seeds on the soaked blotter paper and close the petri dish.

4. Incubate for 7 days at 20°C.

Appendix 2: Multiple comparisons for effect of *A.fabae* on the plant heights, leaf areas and 100-seed weights of the inoculated plants and the control plants.

Multiple Comparisons

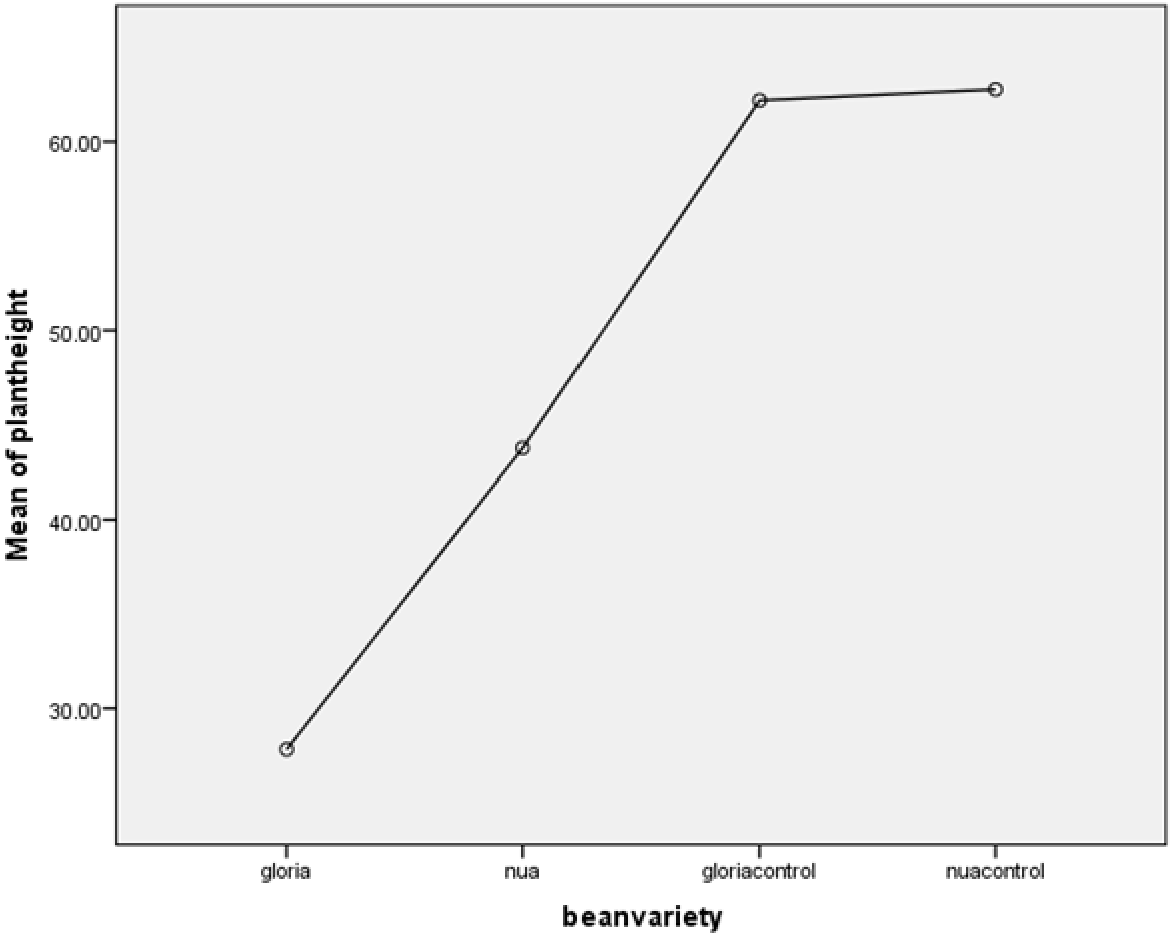
Dependent Variable		(I) beanvariety	(J) beanvariety	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
							Lower Bound
plantheight	Tukey HSD	Gloria	Nua	-15.93417*	.74438	.000	-17.8819
			gloriacontrol	-34.35958*	.74438	.000	-36.3073
			nuacontrol	-34.94208*	.74438	.000	-36.8898
		Nua	Gloria	15.93417*	.74438	.000	13.9864
			gloriacontrol	-18.42542*	.74438	.000	-20.3732
			nuacontrol	-19.00792*	.74438	.000	-20.9557
		gloriacontrol	Gloria	34.35958*	.74438	.000	32.4118
			Nua	18.42542*	.74438	.000	16.4777
			nuacontrol	-.58250	.74438	.862	-2.5302
		nuacontrol	Gloria	34.94208*	.74438	.000	32.9943
			Nua	19.00792*	.74438	.000	17.0602
			gloriacontrol	.58250	.74438	.862	-1.3652
	Dunnett t (<control)[b]	Gloria	nuacontrol	-34.94208*	.74438	.000	
		Nua	nuacontrol	-19.00792*	.74438	.000	
		gloriacontrol	nuacontrol	-.58250	.74438	.416	
leafarea	Tukey HSD	Gloria	Nua	-9.43333*	1.01048	.000	-12.0774

			gloriacontrol	-19.86583*	1.01048	.000	-22.5099
			nuacontrol	-21.46292*	1.01048	.000	-24.1070
		Nua	Gloria	9.43333*	1.01048	.000	6.7893
			gloriacontrol	-10.43250*	1.01048	.000	-13.0765
			nuacontrol	-12.02958*	1.01048	.000	-14.6736
		gloriacontrol	Gloria	19.86583*	1.01048	.000	17.2218
			Nua	10.43250*	1.01048	.000	7.7885
			nuacontrol	-1.59708	1.01048	.395	-4.2411
		nuacontrol	Gloria	21.46292*	1.01048	.000	18.8189
			Nua	12.02958*	1.01048	.000	9.3855
			gloriacontrol	1.59708	1.01048	.395	-1.0470
	Dunnett t (<control)[b]	gloria	nuacontrol	-21.46292*	1.01048	.000	
		nua	nuacontrol	-12.02958*	1.01048	.000	
		gloriacontrol	nuacontrol	-1.59708	1.01048	.136	
Seedweight	Tukey HSD	gloria	Nua	-8.90250*	.88224	.000	-11.2110
			gloriacontrol	-25.05917*	.88224	.000	-27.3677
			nuacontrol	-25.64333*	.88224	.000	-27.9518
		nua	Gloria	8.90250*	.88224	.000	6.5940
			gloriacontrol	-16.15667*	.88224	.000	-18.4652
			nuacontrol	-16.74083*	.88224	.000	-19.0493
		gloriacontrol	Gloria	25.05917*	.88224	.000	22.7507
			Nua	16.15667*	.88224	.000	13.8482
			nuacontrol	-.58417	.88224	.911	-2.8927
		nuacontrol	Gloria	25.64333*	.88224	.000	23.3348
			Nua	16.74083*	.88224	.000	14.4323
			gloriacontrol	.58417	.88224	.911	-1.7243
	Dunnett t (<control)[b]	gloria	nuacontrol	-25.64333*	.88224	.000	
		nua	nuacontrol	-16.74083*	.88224	.000	
		gloriacontrol	nuacontrol	-.58417	.88224	.469	

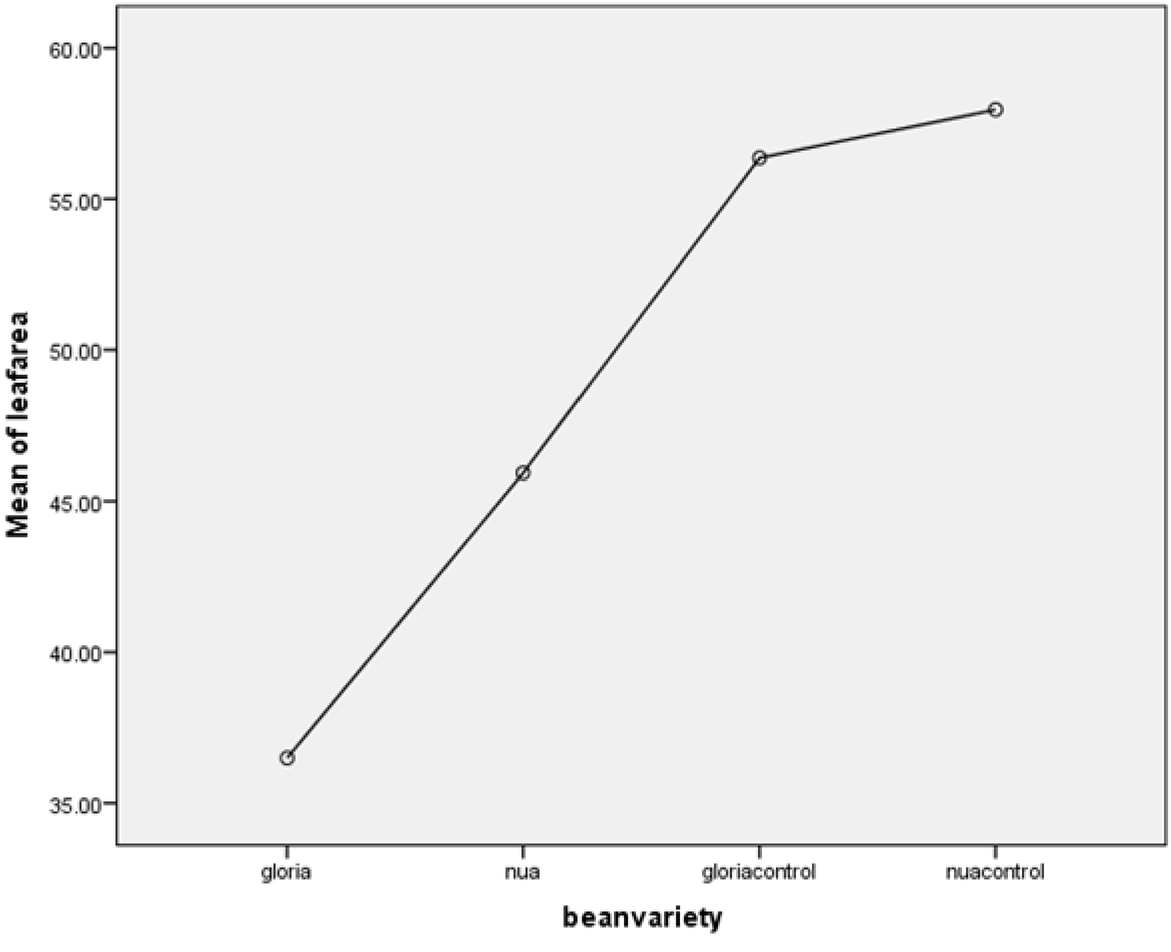
*. The mean difference is significant at the 0.05 level.

b. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Appendix 3: Means plot of the mean plant heights of the inoculated plants and the control plants.



Appendix 4: Means plot of the mean leaf areas of the inoculated plants and the control plants.



Appendix 5: Means plot of the mean 100-seed weights of the inoculated plants and the control plants.