



**INSTITUTO POLITÉCNICO
DE VIANA DO CASTELO**

Iurii Galadzhun

Effect of grafting runner beans on crop yield and fruit quality

Master in Organic Farming

Supervisors
Professor Isabel de Maria Mourão
Doctor Sofia da Rocha Costa

2015

Table of contents

Table of contents.....	2
ABSTRACT	3
LIST OF TABLES	4
LIST OF FIGURES	5
LIST OF ATTACHMENTS.....	7
1. Introduction	8
1.1 Vegetable grafting.....	9
1.1.1 Advantages of grafting	10
1.1.2 Methods of vegetable grafting	11
1.2 Runner beans	14
1.2.1 Runner beans description	14
1.2.2 Runner beans diseases.....	15
1.2.3 Grafting of Runner beans	19
1.2.4 The fruit quality of green beans.....	19
1.3 Objectives of the thesis	20
2. Materials and methods.....	20
3. Results	28
3.1 Dead plants.....	28
3.2 Crop development and growth	29
3.3 Dry matter content of the pods	33
3.4 Pod length.....	34
3.5 Pod defects	36
4. Discussion and conclusions	38
4.1 Crop development and growth	38
4.2 Crop quality.....	39
Bibliography	40

ABSTRACT

Runner bean is a popular vegetable culture. It is often cultivated in green houses. To intensify its production, synthetic fertilizers along with pesticides are often used. However, using grafting can effectively and inexpensively fight root parasites and increase the uptake of nutrients from the soil, due to the development of a stronger root system of the plant. This method has previously shown promising results for tomatoes.

In this work we have studied how grafting of runner beans cultivars Rajado and Oriente on the rootstocks P1: cv. Aintree (Tozer Seeds), P2: cv. White Emergo (TS), and P3: cv. feijão de 7 anos (Portuguese landrace cv.) can affect plant performance, yield and resistance of the crop to soil borne disease, such as nematodes and *Fusarium*.

We recorded the number of days after planting (DAP) at which the first flower and the first pod occurred in each crop treatment. Also we measured the length of the pods, their fresh and dry weights, and the frequency of diseases and defects on the pods. The temperature and relative humidity inside the greenhouse were constantly measured.

During the experiment the plants experienced nutrient deficiency and *Fusarium solani* infection. The plants grafted onto the R3 rootstock, cv. feijão de 7 anos, have shown the best survival rates and yield per plant.

In conclusion, grafting runner bean seems to be an appropriate strategy to increase crop tolerance to soilborne diseases caused by *Fusarium* spp. and nutrient deficiency, mainly for higher yielding cultivars such as cv. Oriente. For the scion cv. Oriente we would recommend the rootstock cv. P3, while for cv. Rajado further investigation is still needed to evaluate the effects of rootstocks P2 and P3.

LIST OF TABLES

Table 2.1 - Experimental soil characteristics, samples taken on 13.02.2015	21
Table 2.2 - Irrigation system work regime	22
Table 2.3 - The plant treatments used	23
Table 2.4 - Plant protection applied	25

LIST OF FIGURES

Figure 1.1 - The vascular tissues (McAvoy, 2015).....	12
Figure 1.2 - Cleft Grafting (Masayuki, 1999)	13
Figure 1.3 - Tongue Approach Grafting (Masayuki, 1999)	13
Figure 1.4 - Tube Grafting (McAvoy, 2015).....	14
Figure 1.5 - The runner beans pods.....	15
Figure 2.1 - Greenhouse of ESA IPVC	21
Figure 2.2 - The areas for soil samples collection	22
Figure 2.3 - Fertilization.....	22
Figure 2.4 - The planting sites in a block	23
Figure 2.5 - The plant treatments used in the experiment	23
Figure 2.6 - The plant treatments used in the experiment	23
Figure 2.7 - The planting scheme	24
Figure 2.8 - Roots infected by <i>Fusarium solani</i>	25
Figure 2.9 - The thermohygrograph at the greenhouse	25
Figure 2.10 - Temperature in greenhouse 22.04.2015 – 2.07.2015	26
Figure 2.11 - Relative humidity in greenhouse 22.04.2015 – 2.07.2015	26
Figure 3.1 - Mean number of dead stems (out of 4) from 68 days after planting, for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.	28
Figure 3.2 - Final number of dead stems for (a) the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants) and for (b) all crop treatments of the cv. Oriente and cv. Rajado. Different letters above bars mean significant differences between crop treatments ($p < 0.05$).	29
Figure 3.3 - Days after planting of the first flower and first pod appearance for (a) the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants) and for (b) all crop treatments of the cv. Oriente and of the cv. Rajado. Different letters above bars mean significant differences between crop treatments ($p < 0.05$).	30
Figure 3.4 - Accumulated number of pods throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.....	31
Figure 3.5 - Total number of pods (m^{-2}) for the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments ($p < 0.05$).	31

Figure 3.6 - Accumulated pods fresh weight (g m ⁻²) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.	32
Figure 3.7 - Crop yield (kg m ⁻²) and pod dry matter (%) for the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments (p <0.05).....	32
Figure 3.8 - Crop yield (kg m ⁻²) for the (a) cv. Oriente and (b) cv. Rajado, grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments (p <0.05).	33
Figure 3.9 - Pod dry matter (%) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.....	34
Figure 3.10 - Mean pod length (cm pod ⁻¹) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3. The line is the linear model for the overall mean pod length in each harvest.	35
Figure 3.11 - Mean pod length (cm pod ⁻¹) for the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments (p <0.05).	35
Figure 3.12 - (a) Crop yield (kg m ⁻²) and mean pod length (cm pod ⁻¹); (b) total number of pods (m ⁻²) and pod dry matter (%), for all crop treatments of the cv. Oriente and of the cv. Rajado. Different letters above bars mean significant differences between crop treatments (p <0.05).	36
Figure 3.13 - Minor pod defects (No of pods m ⁻²) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.	37
Figure 3.14 - Pod defects (minor, severe and with disease symptoms) (No of pods m ⁻²), for (a) the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants) and for (b) all crop treatments of the cv. Oriente and of the cv. Rajado. Different letters above bars mean significant differences between crop treatments (p <0.05).....	37

LIST OF ATTACHMENTS

1. Statistical analysis of the results

1. Introduction

In the period from the 1940s to the 1960s, a revolution in agricultural practices and technologies were introduced globally. Farmers, particularly in developing countries, started to use high-yielding varieties, irrigation infrastructures, pesticides, herbicides, and synthetic fertilizers, which effectively boosted food production in the short term. This process, the **Green Revolution**, was renowned for reducing human hunger in Mexico, India and Pakistan in the 1960s, and this won Dr. Norman Borlaug, the 'Father of the Green Revolution' the Nobel Peace Prize in 1970 (Nobel Prize). But, these rapid advances in agronomy made food production highly intensive and dependent on chemicals and machines; it quickly became market-driven, and failed to take into account local knowledge and culture, and the ecology of agricultural ecosystems (Gliessman, 2013).

Growing a monoculture and placing high artificial selection on more productive plants, over pest and disease resistance, lead to the increase on the occurrence and severity of pests and diseases, with a corresponding yield decrease. In Europe, this varies from 20-35%, depending on the part of the continent (Oerke, 2005). Keeping all the pests and diseases under control using large quantities of pesticides is no longer an option, as these chemicals are often harmful for the consumers and the environment. Until recent time using pesticides, and particularly soil fumigation with Methyl Bromine, was considered one of the main factors for the production success in greenhouses, as an effective way to control soil-borne diseases. But after the **Montreal Protocol** ratification (1987) the use of ozone-depleting chemicals, including **methyl bromine**, was phased out worldwide. The restrictions, made since by the Montreal protocol, have been shown to be effective in protecting the Ozone layer (Mäder, 2010). Methyl bromide was banned in Europe, but a further global prohibition can be expected, which gives an additional market to some alternative pesticides (EPA, 2015), many of which are also toxic and bound to be banned in the next 20 years (Nicol et al., 2010).

A sustainable and ecologically-sound management of the agricultural ecosystems, which builds on scientific knowledge, technology and social-driven practices, must be put in place to prevent pest and disease populations to increase and become more problematic (Gliessman, 2013).

The principles of organic farming, set out in the EU Legislation (EC 834/2007), aim to improve the sustainability of cultivation systems, offer a variety of high-quality products, place a greater emphasis on environmental protection, and give more attention to biodiversity. A concern with human health and social wellbeing is also patent in the EU legislation, in aiming to increase consumer confidence in organic farming and protecting consumer interests.

In organic farming, pest and disease control is done by the use of resistant varieties, biological control, changing the ecological conditions to make it less favorable for pests, using some biological pesticides only when plants are attacked, and by constant monitoring for and removal of infected plants (Hills, 1989). Among these control methods, **biological control** makes use of existing, natural interactions between pests and diseases and their natural consumers, i.e., it is the encouragement of beneficial organisms already existing locally or the introduction of new species

of beneficial organisms to bring down populations of a given pest or disease to a non-damaging level. Fighting the pest by changing the ecological conditions is done by growing **mixed cultures** on one field rather than a monoculture, and crop rotation. It is allowed in organic farming to use the natural products **biological pesticides**. For example extracts from plants, such as pyrethrum and daisy, or bacteria, e.g. *Bacillus thuringiensis*.

The use of **resistant cultivars** is an old and can be an effective method to fight pests and diseases. It is commonly used for horticulture and cereals. However sometimes it is hard to find a resistant cultivar with acceptable yield of desirable characteristics. This control method can have further limitations: the resistant cultivars are frequently only partially resistant and some pests can overcome the resistance. For example, root-knot nematodes *Meloidogyne spp.* can parasitise most of the flowering plants, and, despite their asexual reproduction, can express different parts of their genome to fit the environment, due to high portion of transposable elements in their genome, along with presence of homologous but divergent DNA segments (Castagnone, 2014). Also resistant cultivars can lose their resistance as a consequence of unfavorable environment conditions. For example, three out of eleven studied tomato cultivars, resistant against root-knot nematodes, have significantly increased their susceptibility toward *Meloidogyne arenaria* and *Meloidogyne javanica* after a heat stress. It was found that the *Mi* gene in tomato, which confers resistance to *Meloidogyne sp.* attack, ceases its expression at soil temperatures above 28°C (Verdejo-Lucas, 2013).

1.1 - Vegetable grafting

Where pest and/or disease –resistant cultivars are available, but with limited yield or with no commercial value, these can be used as rootstock cultivars onto which the commercially-producing cultivars can be grafted.

Grafting has historically been done on fruit trees, and has a special value in fruit culture, where the raising of resistant varieties is made difficult by the slow growth of the trees. A good example of the successful usage of grafting for plant protection is the **Phylloxera** pest of grape vine (*Vitis vinifera*). In 1865 all the vine industry of France was threatened by the pest bug Phylloxera (*Phylloxera vitifolii* Fitch). The Californian vine, *V. californica*, is immune to the pest which is now controlled by grafting European cultivars to American rootstocks (Hubert, 1983).

Vegetable grafting, already used in ancient times in Japan, is still very recent in Europe, and has been growing increasingly since the 2000s. It can help vegetable plants to cope with soil-based stresses: not only soil borne pathogens, but also inadequate abiotic conditions (salt, temperature). Industrial application of grafted vegetable plants started in late 1920-ths in Eastern Asia – Japan and Korea. The most popular vegetables for grafting are watermelon, cucumber, melon, tomato and eggplant. Later this technique has been progressively used throughout Europe and worldwide. In Portugal the vegetable grafting was first introduced in tomato plants, where it is estimated that, by 2009, half of the commercial production of fresh tomato was being grown on grafted plants (FLF, 2010). Grafted watermelons, melons and cucumbers are also very common (Rodrigues, 2009). The most popular for grafting vegetables are cucurbits and solanaceous families (Lee et al, 2010).

An interesting field in the science and technology of grafting is the development of **grafting robots**. According to (McClure, 2008), semi- or fully-automated grafting robots were invented by several agricultural machine industries in the 1990s, yet the available models are limited. The lack of flexibility of the existing robots also limits their wider use.

There are very recent articles about improving the efficiency of grafting robots. In the journal of robotics the developed grafting robot was described. It is capable of clipping, moving, positioning, cutting, grafting, and binding saplings. Experiments show that the stock cutting efficiency is 98.4%, the scion cutting efficiency is 98.9%, the grafting efficiency is 87.3%, and the binding efficiency is 68.9%. (Qun Sun, 2014). So, in perspective, the automated grafting can be used to decrease the price of the grafted plants. Currently grafting robots are still not used widely due to pathogen problems: if one of the plants is infected, the robot blade will spread the infection through all the plants it cuts.

1.1.1 - Advantages of grafting

The use of grafting can bring about many advantages. Grafting may be used to **boost plant growth and development**. It was shown that the rootstock genotype determines the yield, and the macronutrients content in the leave of the scion, primary N and Na (Ruiz, 1997). For this reason, the planting **density** of grafted plants could be **half the conventional**, and still provide high yields due to increased plant vigor. Grafted plants usually need **less fertilizer** due to the powerful root system (Rodrigues, 2010). It is recommended to decrease the use of fertilizers by 30% or more for grafted comparing to self-rooted plants, depending of the rootstock cultivar (Lee and Oda, 2003; Salehi-Mohammadi, 2009). Grafting also can help to **extend harvest period** and **increase the yield** (Lee et al, 2010).

Grafted plants also can be used to allow agricultural production in **hot-wet season**. It was shown, that tomatoes grafted on eggplant rootstock can tolerate floods, waterlogged soils, and soil borne diseases much better than ungrafted ones. Using grafted tomatoes can reduce the yield loss or even plants death during floods (Black, 2003). It was also shown, that grafting can be used to increase the plant tolerance to **low temperatures** (Rivero, 2003)). Grafting cucumbers on low temperature resistant rootstock can promote plant growth and early fruit production in suboptimal temperatures in winter (Nijs, 1984).

An exciting outcome of research on vegetable grafting, especially for cultivation in greenhouses, is the use of grafting to obtain plants **resistant to salinity** of the soil. Soil salinity has two separate effects - osmotic or an ion-specific effect, which cannot be fully separated. The osmotic effect is always proportional to salt concentration. The ion-specific effect is based on the fact that different ions can interfere with the uptake of the essential ions e.g. K^+ , Ca^{2+} , Mg^{2+} (Colla, 2012). Salinity can be problematic in greenhouses, as leaching of soil additives is reduced. Besides **NaCl salinity**, there are problems with **NaSO₄ salinity** in parts of India, Egypt and California. In saline conditions, grafted plants can perform much better than ungrafted ones by many parameters, such as shoots nutritional status and net assimilation rate. Thus far, grafting is more effective against NaSO₄ salinity than against high NaCl levels (Colla, 2012). In his article, Dimitrios Savvas shows, that grafting tomatoes on commercial rootstocks (Beaufort, He-Man, Resistar) can prevent a yield loss at low (22 mM NaCl) and moderate (45 mM NaCl) soil salinity levels. The plants grafted onto

two of the tested rootstocks gave higher yields only in comparison with the nongrafted plants, and the differences were significant only at low (Beaufort) or moderate (Resistar) salinity. Yield differences between grafting treatments at low and moderate salinity arose from differences in fruit number per plant, while mean fruit weight was not influenced by grafting or the rootstock. NaCl salinity had no effect on the yield of plants grafted onto He-Man but restricted the yield in all other grafting treatments due to reduction of the mean fruit weight. Grafting onto the three tested commercial rootstocks significantly reduced the leaf Mg concentrations, resulting in clear Mg-deficiency symptoms 19 weeks after planting (Savvas, 2011). Using grafting to improve the salinity resistance is developed for such vegetables as tomatoes, cucumber, squash and bottle gourd, melon, watermelon, eggplant (Rouphael, 2010).

Grafting also affects the **quality of fruits**. If on one hand it can increase fruit quality and increase the yield of commercial fruits, change their colour and increase their size, on the other hand the rootstock can also affect their taste: in a so far ill-explained phenomenon, watermelon plants grafted onto pumpkin can produce watermelons with a hint of cucurbit taste (Lee, 2003). In fact, in 2012 it was proven that genetic information may migrate from rootstock to scion through grafting. Transfer of entire chloroplasts, or at least their genomes, can occur in contact zones between plants. The new chloroplast genome can even be handed down to the next generation and, thereby, result in a plant with new traits. These findings are of great importance to the understanding of evolution as well as the breeding of new plant varieties, and above all to grafting itself (Stegemann, 2012).

Grafting can provide multiple **advantages over self-rooting** in some plant species, other than vegetables. Grafted scions provide larger numbers of plants compared to rooted cuttings and more shoots can be taken from the donor parents for grafting than for cuttings. The significantly reduced time to establish grafted scions compared with rooted cuttings is another advantage of grafting compared with rooting. Grafting could potentially help with the **propagation and perpetuation of sterile or recalcitrant interspecific hybrids**, and could also be used to increase seed multiplication in determinate type beans (Gurusamy, 2010).

Importantly, grafting has been described as an effective and eco-friendly way to control **soil borne diseases** in tomatoes, watermelons, melons, cucumbers, peppers, eggplants, and other horticultural plants. The former include: fungal pathogens *Verticillium*, *Fusarium*, *Pyrenochaeta* and *Monosporascus*; oomycete pathogens like *Phytophthora*; bacterial pathogens, particularly *Ralstonia*; root knot nematodes *Meloidogyne* sp. and several soil-borne virus pathogens. fusarium wilt, bacterial wilt, verticillium wilt, monosporascus root rot, and nematodes” (Louws, 2010). Grafting can also increase tolerance to foliar fungal diseases, viruses, and insects (King, 2008). This makes the use of grafted plants appealing also for **hydroponics** farming, as in such systems there are high chances of rapid spread of diseases (Lee et al, 2010).

1.1.2 - Methods of vegetable grafting

For successful grafting the scion and the stock should be compatible and produce a single, functioning plant. When two pieces of stem tissue fuse together in growth from the meristematic area, there is a line, or point, or plane at which the adjacent cells originated from two different

sources. The xylem and phloem tissues on either side of this line should be similar enough to permit the passage of nutrients and water through the graft union. Incompatible grafts will sometimes fuse and grow, but normal development is restricted to some extent.

Before grafting it is important to make sure that the grafted plants are turgid. When grafting in greenhouses it is better to graft in the morning or in the evening to avoid water stress. The place for grafting should be shadowed and not windy (McAvoy, 2015). Maintaining the moisture content of rootstock and scion is an important rule for success in producing grafted plants. In the aerial parts of the plant, water loss is prevented by using a grafting compound or rubber tape. Placing in an atmosphere of 100 percent relative humidity prevents water loss from grafted herbaceous plants.

The point of the graft should be above ground level, so that the commercial variety does not send roots and soil-borne diseases are handled by the rootstock. However, the graft needs to be done below the rootstock cotyledons, as this prevents the occurrence of **suckering** - rootstock shoot regrowth (Bausher, 2011).

Both the scion and the rootstock should be cut at the same angle to promote a full contact between the rootstock and scion. Accordingly, the diameter of the scion and the rootstock should be approximately equal at the union point and they should be in tight contact before clipping is put in place, to match all the vascular tissues (McAvoy, 2015). For successful grafting it is very **important to match all the plant's vascular tissues** (Fig. 1.1). The **Xylem** provides the movement of water and nutrients from the roots to the top of the plant. The **Phloem** distributes sugars and hormones produced in the leaves throughout the plant. The **Cambium (meristem)** is where the stem growth in diameter takes place. Finally, the union of the two plant parts should be done as quickly as possible after cutting, as waiting for more than a few minutes after the cut was done can hinder success.

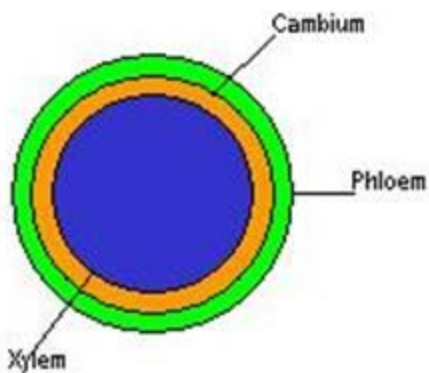


Figure 1.1 - The vascular tissues (McAvoy, 2015)

It is important to provide proper **healing and acclimatization** for grafted plants in order for them to survive. The grafted plants should be kept in relative humidity of 95% or more for 3-5 days until the cut ends knit together, or heal. Usually the grafted plants are placed in a special tent for **shadowing**. Pressure **sprayers** are often used for misting twice a day to provide proper humidity (McAvoy, 2015).

Cleft Grafting

For grafting with the cleft graft methods the rootstock should be sown 5-7 days prior to the scion plants. When the rootstock reaches the 5-6 leaves stage, the stem from both the scion and the rootstock with 2-3 leaves remaining are cut. The scion is cut in a wedge shape and placed into the cleft cut in the end of the rootstock. Then the graft is held firmly with a clip (Fig. 1.2) (McAvoy, 2015).

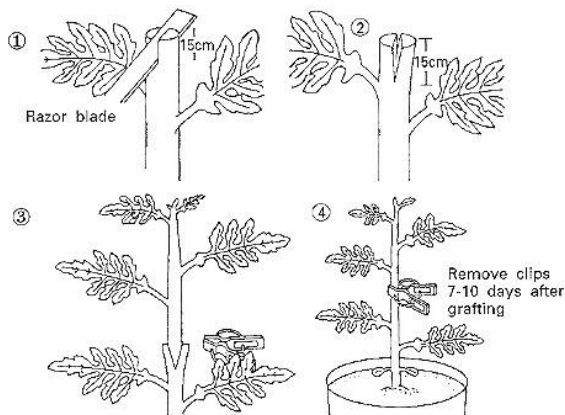


Figure 1.2 - Cleft Grafting (Masayuki, 1999)

Tongue Approach Grafting

This technique provides high grafting success rates, and because of this it is often used to graft melons, cucumbers, and other Cucurbitaceous species. It is also used for tomatoes, when there are no facilities to provide good healing conditions.

To perform this method older plants are needed, with sufficient stem diameter. The top of the rootstock is removed, then both the scion and the rootstock are cut in a tongue shape, connected and fixed with a larger clip. After healing for 3-4 days the scion is cut off from its original root and left on the rootstock (Fig. 1.3) (McAvoy, 2015).

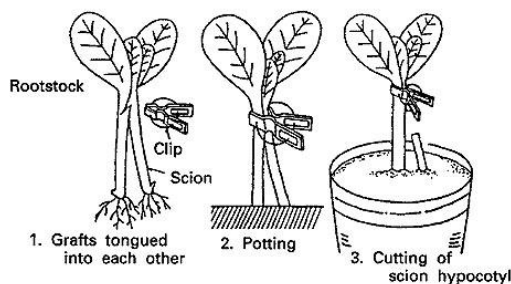


Figure 1.3 - Tongue Approach Grafting (Masayuki, 1999)

Besides the high success rate of plant union, approach grafting provides the lowest rate of rootstock shoots developing in the field as compared to other grafting techniques. However it is more time-consuming and laborious than other grafting methods.

Tube Grafting

For this grafting method the rootstock should be sown 1-2 days before the scion. Tube grafting is suitable for young plants. The grafting speed of this technique is 2-3 times faster than with the cleft grafting and grafted plants require less space in the healing facilities.

It is good to start grafting when the scion has developed one pair of true leaves. Before grafting the tubes with the diameter that matches the stems diameter should be chosen. Then the rootstock should be cut with a razor blade at 30-45°. The cut should be done **below the cotyledons**. **Otherwise** there is a risk of **the axillary buds** development. Then a tube should be placed on the rootstock on a half of its length. After that the scion is cut at the same angle as the rootstock and then placed in the tube in tight contact with the cut on the rootstock.

It is important to mist the grafted plant to prevent desiccation. Healing takes 4-7 days, and after healing the scion part of the grafted plant often is **pinched** to develop two **axillary shoots**. As with other grafting techniques, it is important that the diameters of the scion and the rootstock are approximately the same size. Also the tube should be chosen to match the diameter of the stems (Fig. 1.4) (Kubota, 2015).

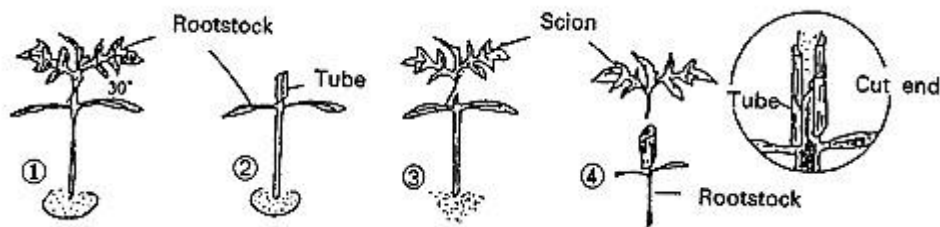


Figure 1.4 - Tube Grafting (McAvoy, 2015)

1.2 - Runner beans

1.2.1 - Runner beans description

Runner beans (*Phaseolus coccineus*, feijão-verde) is a plant in the *Fabaceae* family (previously Leguminosae) or the bean family. Although it is mainly grown for food, there are also some ornamental cultivars. Like other members of legume family, the bean plant forms associations with nitrogen fixing bacteria (rhizobia) in its roots. The bean is day neutral – its flowering and pod set are unaffected by day length.

This species originated from the mountains of Central America. Most varieties have red flowers and multicolored seeds, though some have white flowers and white seeds. The vine can grow to 2 m, and in some cultivars – as long as 4 meters. Bush types exist, dwarfness being an inherited trait. The beans from non-climbing bush or dwarf runner beans are considered lower-quality comparing to the ones produced by the climber cultivars. However the non-climbing beans are easier to harvest (RHS, 2015). The root system has a thick taproot and is fibrous. The knife-shaped pods are normally green. Protein content of the mature seed is about 17%, with carbohydrate content about 65%.

The species is perennial, but it is usually grown in production systems in Europe as an annual. Like other *Phaseolus*, runner beans are frost-sensitive, and the optimal mean daily temperature remains between 15° and 30° C. Temperatures below or above the range can have deleterious effect on the performance of the bean plant. Below 0° C and above 35° C germination does not occur, whereas in the appropriate temperature range, beans will germinate in under six days. Production of fresh pods is more possible in cold climate than seed production. Windy and rainy climate is unfavorable, because it increases spread of foliar diseases (Nonnecke, 1989).

For proper development, runner beans need large amounts of water, especially if the weather is dry when the buds start to appear. It is recommended to apply 5-9 liters of water per square metre every three to four days during the farming season. When the bean shoots have reached the top of their support they are usually pinched out to prevent plants from becoming top heavy (RHS, 2015).

Plant spacings are about 15 to 30 x 90 cm for bush-type cultivars, and about 15 to 30 x 150 for pole- or trellis-supported production. About 4 months are required to complete the annual growth cycle. In Central America the crop may be interplanted, usually with maize.

The seeds of the plant can be used fresh or as dried beans. The pods are edible whole while they are young and not yet fibrous. The starchy roots are still eaten by Central American Indians. In some regions flowers are also consumed (Rubatzky, 1997).

During the cold season, the pods of the runner beans sold as "green beans", "snap beans" or "string beans" in European markets usually come from Greece and northern Africa. The pods of runner beans can be identified by their big size and rougher surface.

Runner beans are a popular ingredient in Portuguese, Spanish, Greek, Austrian, and partially in German cuisine.



Figure 1.5 - The runner beans pods

1.2.2 - Runner beans diseases

For French, Navy and Runner beans the most common diseases are: Anthracnose (*Colletotrichum lindemuthianum*), Botrytis Pod Rot (*Botrytis cinerea*), Foot rots (*Fusarium solani*), Fusarium Wilt (*F. oxysporum f. sp. phaseoli*), Halo Blight (*Pseudomonas phaseolicola*), Rust (*Uromyces appendiculatus*), Sclerotinia Disease, Bean Yellow Mosaic Virus and Bean Common Mosaic Virus (House, 1982).

Anthracnose can lead to pronounced losses in beans. It is caused by the fungus *Colletotrichum lindemuthianum*. The infection is usually introduced by infected seeds and spread onto other plants with spores via rain drops. A heavy rainfall may spread the spores as far as 4.5 meters from the initial host plant (Pynenburg, 2011). The infected plant has reddish-brown spots on its pods, stems, veins, and under the surface of the leaves. The disease can be managed by checking the seeds for fungus, using fungicides on seeds, and burning plants in the end of season.

Botrytis Pod Rot is caused by the mold fungus *Botrytis cinerea*. Botrytis is a wide-spread pest of grapes, strawberries and some other plants. It is dangerous for runner beans mostly in the wet season, usually when the pods are touching the soil. The infected plant will have an identifiable a grey mold on its pods. To fight the disease the plants should be provided with a good drainage.

Halo Blight (*Pseudomonas phaseolicola*), causes water-soaked spots with yellow halo on affected leaves and pods. This seed-borne bacterial disease can result in severe epidemics in wet and cold summers. The infection spreads through wind-driven rain. Using infected seeds may result in plants with no growing point, so-called “snake heads”. Use of colloidal copper or copper oxychloride may stop the spread of infection. It is recommended to check the seeds for halo blight at the seed testing stations.

Rust (*Uromyces appendiculatus*) is not a very common bean disease. The incidents of the infection are reported occasionally. The symptom is the brown or black pustules, with spores inside, appearing on the leaves. To control the disease it is recommended to burn or bury infected debris.

Sclerotinia Disease is caused by fungus *Sclerotinia sclerotiorum*. It is common in both outdoors and protected bean crops. The disease is favored by temperatures above 22°C. The fungus survives in soil, grows on debris, producing cup shaped bodies which give rise to airborne spores. The disease attacks the main stem of the bean which result in plant's wilting and collapse (House, 1982).

Bean Yellow Mosaic Virus and Bean Common Mosaic Virus is spread by **aphids** and rarely can be transmitted by seeds. The symptoms are greatly dependent on virus strain and on cultivar of the host plant.

Bean Common Mosaic Virus transmits in seeds and is spread by aphids. The main symptoms are leaves becoming crinkled and mottled with dark and pale green areas. To avoid the spread of the disease it is recommended to eliminate the infected plants and apply the means of aphid control.

Soil-borne diseases

Fusarium Wilt (*F. oxysporum f. sp. phaseoli*) is a vascular fungal disease which can be very dangerous for runner beans. *Fusarium oxysporum* spreads by water splash and planting equipment on short distance, and by infected transplants and seed – on long distance. It infects plants by means of mycelia or by germinating spores penetrating the plant root tips. The mycelium penetrates in the xylem vessels and produces microconidia (asexual spores). Eventually the mycelium clogs the vascular vessels and causes the plant death. At first the leaf edges of the infected plant turn yellowish-green, and then the whole leaf becomes dry. To control this disease it is recommended to promote drainage of the soil if it is too wet, perform crop rotation and use treated seeds (House, 1982). The typical symptoms of the disease are yellowing and eventual

browning of the lower leaves and also brown staining of the xylem when exposed with a knife (Adams, 1984).

Nematodes

Nematodes, traditionally known as **eelworms**, are an important part of the soil microfauna, and are the most abundant and diverse animals in soil (there may be 30 million per square meter). While most nematodes are harmless or even beneficial (involved in decomposition processes), plant parasitic nematodes can directly impact the plant by feeding on its roots, and indirectly by affecting root architecture, interfering with water use efficiency, and facilitating infection by secondary pathogens, including bacteria, fungi and viruses (Costa et al., 2011). In a healthy soil, plant-parasitic nematodes are usually kept under control by fungi, bacteria and other nematodes. However they sometimes build up in the soil to the point where the soil becomes "sick." Plants growing in such soil show signs of nutrient deficiency because of serious root damage." (SmartGardenerInc., 2014)

Jones et al. have studied the economic effects of different pathogenic nematodes and published the list of the top 10 most pathogenic plant-parasitic nematodes: "(1) root-knot nematodes (*Meloidogyne* spp.); (2) cyst nematodes(*Heterodera* and *Globodera* spp.); (3) root lesion nematodes(*Pratylenchus* spp.); (4) the burrowing nematode *Radopholus similis*; (5) *Ditylenchus dipsaci*; (6) the pine wilt nematode *Bursaphelenchus xylophilus*; (7) the reniform nematode *Rotylenchulus reniformis*; (8) the needle nematode *Xiphinema index* (the only virus vector nematode to make the list); (9) the false root-knot nematode *Nacobbus aberrans*; and (10) *Aphelenchoides besseyi*." (Jones et al, 2013).

Root Knot Nematodes (*Meloidogyne* sp.) are obligate parasites of the roots of wide range of plant species that are very common in soil. The disease can be lethal for young plants and reduces yields in older ones. Nematodes cause an estimated 5% of the world yield loss (Sasser, 1985), but some infested fields can be abandoned as they are notoriously difficult to control (Nicol et al., 2010). The nematode *M. incognita* is probably the most damaging pathogen in the world (David, 2001).

Nematode species can be found in different climates from frozen soils to rainforests and remote islands (Sasser, 1985). *M. incognita*, which is one of the harmful species of nematodes, can survive only in places with mild winter, with average January temperature -1.1 °C or above. Like all nematodes, root-knot nematodes depend on soil moisture and live in water films in soil. Eggs and even the infective larvae, a resistant or dauer stage, can die in dry soil. To survive they need enough soil water to keep soil humidity at near 100% humidity (Taylor, 1978). In farms with sandy well-drained soil, these nematodes have been reportedly more problematic (Hagedorn, 1986).

There are approximately 100 species of root-knot nematodes, and of these only four have been given worldwide importance: *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla*. The root knot nematodes are usually no bigger than 0.5 mm in length and 0.015 mm in width and are too small to be seen with naked eye. They can be difficult to identify, and an analysis of their morphology will often need to be complemented by a biochemical or molecular analysis. As many, yet not all, species of root-knot nematodes are polyphagous, it is key to determine which species of root knot nematodes infects the crop, to plan the crop rotation (Queensland, 2015).

Root-knot nematodes do not produce any specific symptoms in the part of the plant above the ground, but show symptoms or root failure. Affected plants may look weak and often show symptoms of wilting or chlorosis (yellowing). Below ground, root-knot nematodes cause quite distinctive symptoms, like lumps, galls from 1 to 10 mm or more in diameter, all over the roots. In severe cases roots may rot away (Queensland, 2015).

Root-knot nematodes spend most of their life cycle inside plant roots and are very difficult to control. There are a few groups of methods to fight nematodes: crop rotation, biological control, chemical control, resistant cultivars, and solarization.

Crop rotation is helpful against many pests and diseases. To effectively exterminate nematodes it is good to rotate vegetable with grain cultures, but this also depends on the root-knot nematode species present (Hagedorn, 1986).

Biological control of nematodes is introducing their natural enemies into the field. For example *Pasteuria penetrans* – bacteria, that is an obligate parasite of root knot nematodes (Charles, 2005). Also various trapping fungi, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Hirsutella* spp., some antagonistic rhizosphere bacteria, and endophytic fungi - non-pathogenic root-infecting fungi and mycorrhizae, can be used to fight nematodes (Kerry, 1997). For biological control a **trap crop** also can be used. This crop is planted near the main crop at the important stage of the pest life cycle, ie, when second stage larvae are moving in the soil. The trap crop attracts this infective stage that enters its roots and is then removed before they can multiply and move to the main crop (Wesemael, 2011).

Chemical control consists of the application of pesticides to kill nematodes. The most popular pesticides for this purpose are various Organophosphates and Carbamates in granular form. Some fumigants, such as chloropicrin and ethylene dibromine now are forbidden to use (Gowen, 1997), and it is estimated that many of the currently used nematicides will be banned in the next few years. The most efficient nematicide was also methyl bromide, which as described above, has already been banned in Europe.

There are some **cultivars resistant** against root-knot nematodes. However, nematodes can often overcome the resistance (Castagnone, 2014). Also, depending on environmental conditions, resistant plants may become susceptible (Verdejo-Lucas, 2013). Besides this, the cultivar might be resistant against one type of nematodes and at the same time susceptible towards other types of nematodes (Wesemael, 2011). Cultivars high resistance against nematodes and/or insects are rare among beans (Singh, 2010).

Soil Solarization is a method to control soilborne diseases, which uses sun heat to produce pronounced cycles of high-low temperatures and kill or attenuate the pathogens. The heating is achieved by covering the soil with plastic for 4 to 6 weeks during a hot period of the year. The top 15 cm of the soil will heat up to as high as 60°C, depending on the location. The plastic allows the heat from sun to stay in the soil. This can kill pathogens, such as nematodes, for 30 – 46 cm in depth, whilst providing conditions for the increase in populations of beneficial organisms (Elmore, 1997).

Deep plowing also has can help to exterminate nematodes, albeit with limited success (Hagedorn, 1986).

1.2.3 - Grafting of Runner beans

According to the available information, grafting of runner beans can be used to control soil borne diseases, caused by *Fusarium* spp. and *Meloidogyne* spp. in greenhouse crops. However it can cause negative effect on crop growth and development on all grafted, compared to ungrafted plants (Mourão, 2014). Also, it was shown by Mullin and Abawi that the resistance of bean plants against nematodes can be contributed solely by the root tissues, ie, the grafted plant has the level of resistance conferred by the rootstock. The tested grafted plants with a susceptible rootstock were susceptible towards nematodes, independently of the scion. In the same way, the resistant rootstock provided resistance for the plant, independently of the scion cultivar (Mullin, 1991).

In other studies, an increase in yield was achieved by grafting runner beans as rootstocks, and other *Phaseolus* as scions. Four genotypes of *Phaseolus vulgaris* and one genotype of *P. coccineus* were used as rootstocks. Two genotypes of *P. vulgaris*, and one each of *P. acutifolius* and *P. angustissimus*, and an interspecific hybrid of *P. acutifolius* x *P. angustissimus* were used as scions. Grafting resulted in 91% and 66% higher mean seed yield per plant compared to ungrafted control and rooted cutting treatments, respectively.

Also the common **bean genotype ICA Pijao** was recommended as the best rootstock among the tested rootstocks: *P. coccineus* - Runner bean, *P. vulgaris* - Black bean ICA Pijao, *P. vulgaris* - Pinto bean, *P. vulgaris* - Brown bean, *P. vulgaris* - Pinto bean 1533-15 (Gurusamy, 2010).

According to Rodrigues, a grafted green bean plant costs € 0.50, while a conventional one only € 0.08. However the grafted ones are more vigorous and need less fertilizer due to the powerful root system, can be resistant to *Fusarium* spp., increase yield and production period. The first trials with grafted plants were held in summer of 2008 and produced exciting results, with the authors estimating that 80% of the green beans production in Portugal will be produced using grafting (Rodrigues, 2010).

1.2.4 - The fruit quality of green beans

Green beans should be **harvested** when they are in the rapid development, typically 8-10 days after flowering. To be harvested the pods must be bright color and fleshy, with small green seeds inside. If harvested too late, the pods lose flexibility and become pale green or yellow. The beans should be cooled down as soon as possible after harvesting. Otherwise they will lose water. After the pods lose 5% of their mass they become noticeable wilted. After 10-12% of weight loss the green beans become unmarketable (Cantwell, 2013). It is better to store the beans in a film wrap to maintain high relative humidity and prevent water loss. The **storage** temperature greatly affects the shelf life of green beans. It has been shown that the optimal temperature to store green beans regarding the shelf-life and fruit quality is 10°C. The average shelf life vary between different cultivars (Yagiz, 2010). Often runner beans are stored frozen and have to be thawed prior to commercialization. The thawing process is determinant for fruit quality as the pods lose sensory and nutritional value if thawed at room temperature in polyethylene packaging. The room temperature for thawing in insulation packaging can increase the thawing time for up to 190% and

help to preserve the nutritional value, however the sensory value (taste, color) will still be greatly decreased. The recommended way to thaw green beans is at a temperature close to 0° C: this allows the preservation of nutritional and sensory value of the beans, whilst keeping them safe microbiologically (Martins, 2004).

The **fruit quality evaluation** usually includes the percent of dry mass fraction, the average length of the pods, defects including spots, browning and shriveling, the fruit color, the pod firmness. The fruit color can be estimated by naked eye, but for more precise measurements a colorimeter can be used. The pod firmness is normally evaluated manually, by slightly pressing the pod with fingers. The **laboratory studies** of the fruits usually include the following parameters: pH and titrable acidity, soluble solids content, ascorbic acid content, and chlorophyll content (Yagiz, 2010).

1.3 - Objectives of the thesis

Runner bean is a popular vegetable culture. It is often cultivated in green houses. To intensify its production, synthetic fertilizers along with pesticides are often used. However, using grafting can effectively and not expensive fight root parasites and increase the minerals uptake from the soil, due to development of stronger root system on the plant. This method has previously shown promising results for tomatoes.

The objective of this work is to test how grafting of runner beans cultivars Rajado and Oriente on the rootstocks P1: cv. Aintree (TozerSeeds), P2: cv. White Emergo (TS), and P3:cv. feijão de 7 anos (Portuguese landrace cv.) can affect the yield and resistance of the crop to soil borne disease, such as nematodes and Fusarium.

2. Materials and methods

Grafted runner beans (*Phaseolus coccineus*) were grown under unheated greenhouse conditions during spring/summer 2015, in greenhouse of Escola Superior Agrária, Politechnic Institute of Viana do Castelo (ESA IPVC), located in Refóios do Lima, Portugal (43°38'19.39"N, 16°14'28.86"W) (Fig. 2.1).



Figure 2.1 - Greenhouse of ESA IPVC

The plants were grown in a Cambisol soil with sandy loam texture (Table 2.1). The field was divided in two squares. To see on which square each planting block is located please refer to figure 2.2.

Table 2.1 - Experimental soil characteristics, samples taken on 13.02.2015

	pH	EC	OM	P ₂ O ₅ ER ^a	K ₂ O ER ^a	Ca	Mg
	H ₂ O	(dS m ⁻¹)	(g kg ⁻¹)	(mg kg ⁻¹)			
Square 1	5.8	0,168	49	49	86	113	29
Square 2	5.9	0.136	45	51	80	24	51

(a) ER - Egner-Rhiem method

For this culture we used three different fertilizers. On February 18th, 21 days before planting, we applied organic fertilizer, made of **sheep and cow manure** mixed with corn husks and stored/composted in piles for 5-6 months, at rate **16 t per hectare** (Fig. 2.3). After this, a commercial organic fertilizer **Organihum Nitro-Plus** was applied. This product consists of amino acids obtained from enzymatic hydrolysis of vegetable matter. It was applied through irrigation system for one watering, at concentration 150 ml/hl on 1.04.2015, and at 200 ml/hl on 8.04.2015, 24.04.2015, 30.04.2015, 8.05.2015, and 5.05./2015. A conventional fertilizer **Nitromagnesium** was applied on 1.06.2015 at rate 5.18 kg/ha. The fertilizer is a homogenous mixture of NH₄NO₃, CaCO₃ and MgCO₃. It contains 27% of Nitrogen, 3.5% of CaO and 3.5% MgO.

The soil was covered with plastic film. The **irrigation** was performed by a drip system, with drippers spaced at 0.30 m with 5.42 l*h⁻¹ flow rate (Table 2.2). The total use of water for the experimental plants was 3544.68 l = 59.078 l * 30 blocks * 2 planting sites

Table 2.2 - Irrigation system work regime

Dates		Duration, days	Irrigation, min/day	Per one hole		
From	To			Flow rate, ml/min	Water per day, ml/day	Water total, ml
11-03-2015	22-03-2015	12	2	90.3	180.7	2 168
23-03-2015	19-04-2015	28	4	90.3	361.3	10 117
20-04-2015	02-07-2015	74	7	90.3	632.3	46 793
114						59 078

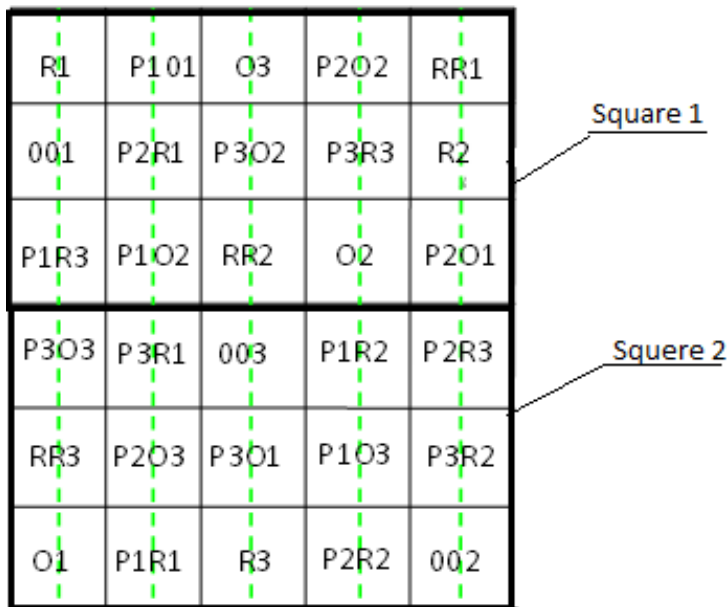


Figure 2.2 - The areas for soil samples collection



Figure 2.3 - Fertilization

A randomized block design experiment with **three repetitions** and **ten treatments** was carried out to evaluate crop yield and quality.

Each one of **thirty blocks** had 7 **planting sites**. For the experiment we used only the two plants in the middle of each block, called the test plants. The other four plants on the sides of the tested plants were used as “guardians” (Fig. 2.4).

G	G	T	T	G	G	X
---	---	---	---	---	---	---

G – Guardian plants

T – Tested plants

X – Empty spot

Figure 2.4 - The planting sites in a block

As the scions we used bean cultivars O:Oriente and R:Rajado. As the rootstocks we used: P1: cv. Aintree (TozerSeeds), P2: cv. White Emergo (TS) and P3:cv. feijão de 7 anos (Portuguese landrace cv.). Also, for control experiment, we used self-grafted plants: OO and RR, and ungrafted plants: R and O (Table 2.3).

The plants grafted on the rootstocks **P1, P2 or P3** originally had **2 stems** per plant. So they were planted one plant per one planting site. The **self-grafted** and **ungrafted** plants originally had **one stem** per plant, and they were planted two plants per one planting site (Fig. 2.6).

The plants were kindly provided by Dr. Rui Gilberto Calico, th manager at Viveiros Novos company, which is located in Povoá do Varzim (Fig.2.5).



Figure 2.5 - The plant treatments used in the experiment



Figure 2.6 - The plant treatments used in the experiment

Table 2.3 - The plant treatments used

#	Treatments	Code	Nº of plants
1	Grafted P1 x O	P1O	18
2	Grafted P1 x R	P1R	18
3	Grafted P2 x O	P2O	18
4	Grafted P2 x R	P2R	18

5	Grafted P3 x O	P3O	18
6	Grafted P3 x R	P3R	18
7	Grafted O x O	OO	36
8	Grafted R x R	RR	36
9	Ungrafted O	O	36
10	Ungrafted R	R	36

O: Oriente **P1: cv. Aintree (TozerSeeds)**

R: Rajado **P2: cv. White Emergo (TS)**

P3:cv. feijão de 7 anos (Portuguese landrace cv.)

The beans were grown at a stem spacing of 0.3 m in the line and 2 m between lines, in a plant density of 0.334 and 0.667 plants per m² respectively for the 1 and 2-stemmed plants (Fig. 2.7).

The training system was 2 m high and made of a two nylon strips per a planting site. After reaching the top of the training system the plants were pinched.

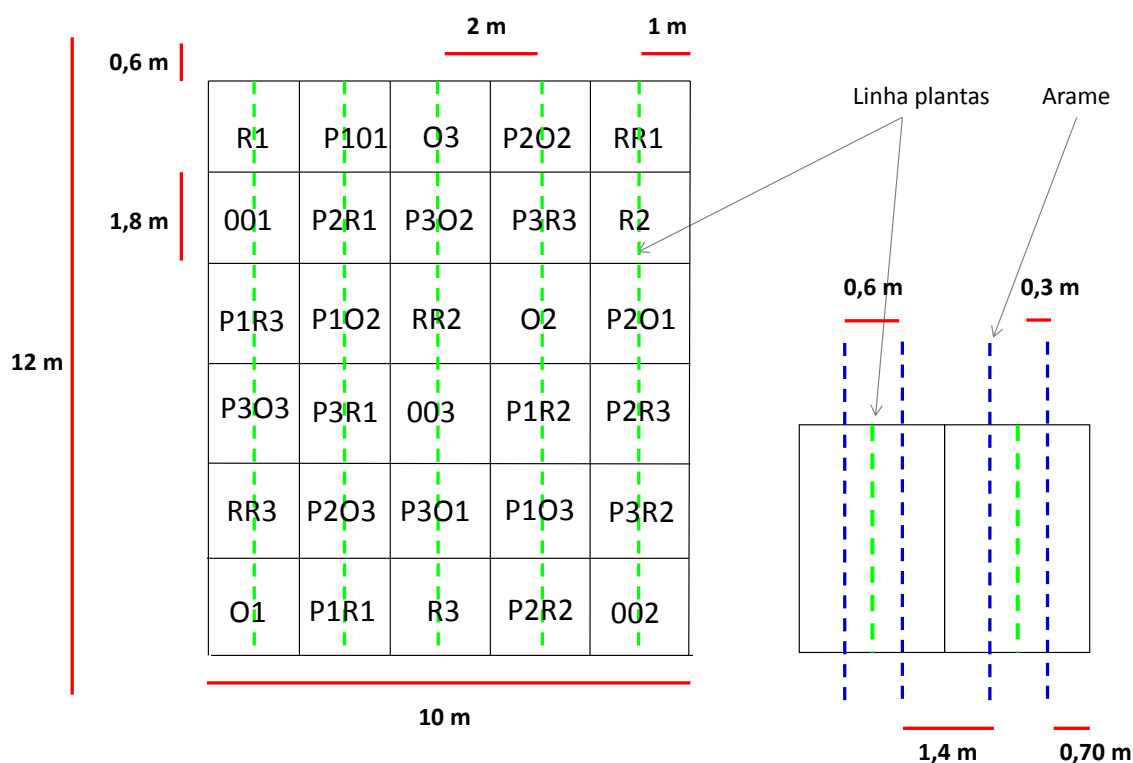


Figure 2.7 - The planting scheme

Various plant protection products were applied (Table 2.4).

Table 2.4 - Plant protection applied

<i>Pest/disease</i>		<i>Product's commercial name</i>	<i>Active substance</i>	<i>Dose</i>	<i>Date</i>
<i>Common name</i>	<i>Scientific name</i>				
Black legume aphid	<i>Aphis craccivora</i>	-	Nettle extract	1Kg/10 l of water	13.05.2015
Fusarium (Fig. 2.8)	<i>Fusarium oxysporum and Fusarium solani</i>	Previcur	Fosetyl sodium - 310 g/l or 27,68% m/m and propamocarb hydrochlorite - 530 g/l or 47,32% m/m	150 ml/ hl	3.06.2015 and 17.06.2015
Thrips	<i>Thysanoptera</i>	Rufaste	75 g / l or 7.02% m/m acrinathrin	80 ml/ hl	3.06.2015
Silverleaf whitefly	<i>Bemisia argentifolii</i>	Fastac	100 g / l or 10.95% m/m alpha-cypermethrin	30 ml/hl	29.06.2015



Figure 2.8 - Roots infected by *Fusarium solani*

The temperature and relative humidity of the air inside the greenhouse were measured and recorded by the thermohygrograph (Fig.2.9). The temperature and relative humidity values were recorded during the period from 22.04.2015 till 02.07.2015 (Fig. 2.10 and 2.11).



Figure 2.9 - The thermohygrograph at the greenhouse

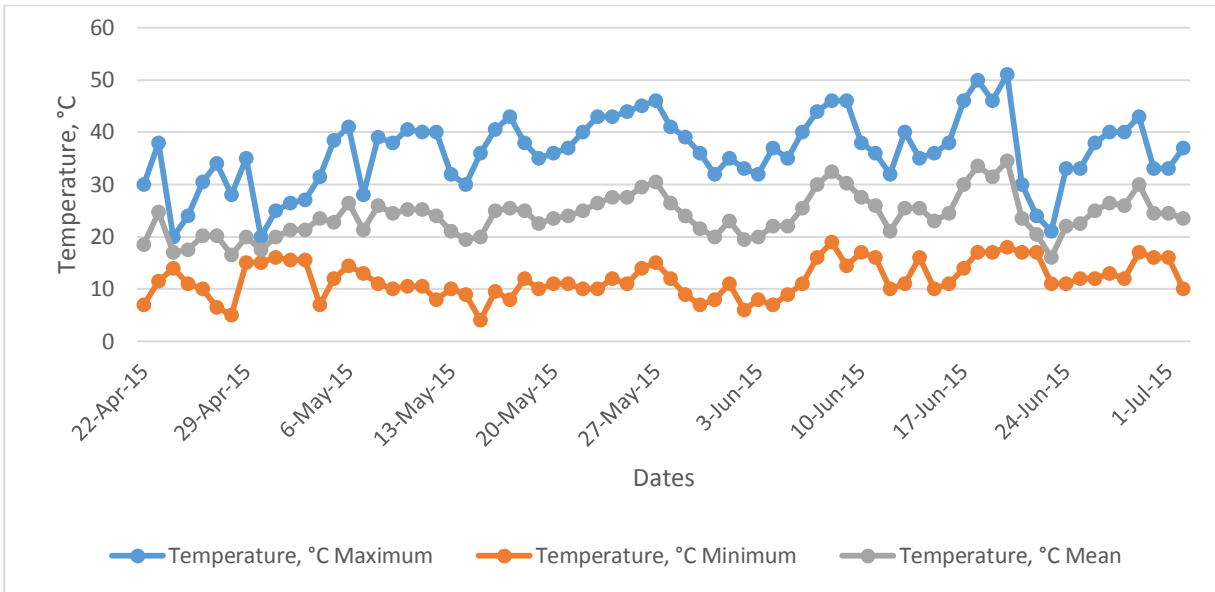


Figure 2.10 - Temperature in greenhouse 22.04.2015 – 2.07.2015

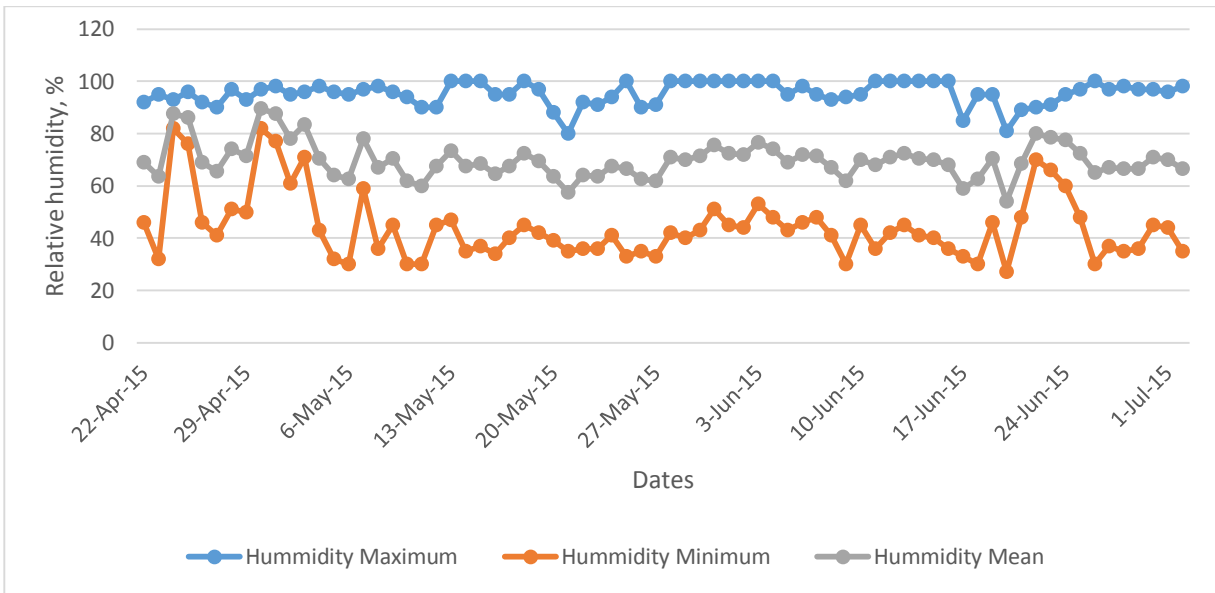


Figure 2.11 - Relative humidity in greenhouse 22.04.2015 – 2.07.2015

The pollination was done by bees, naturally visiting the greenhouse through the ventilation. Weeding was done by hands, no herbicides were applied.

The bean plants were **planted** in the greenhouse on **March 11th**. The first **harvest** took place on **May 6th** (56 days after planting) and the last harvest on July 2nd (114 days after planting). During these 57 days two plants for each treatment repetition were **harvested two times per week** (16 harvests). The number and length of the bean pods, the number of pods with minor defects and number of pods with serious defects, as well as fresh weight were recorded for each harvest. Dry weights were evaluated for four harvests during the harvesting period. Fruit dry matter content was determined after drying the fruit in a ventilated oven at 70°C for 48 hours.

Analysis of variance (ANOVA) was performed by the general linear model SPSS procedure using SPSS 17.0 for Windows (SPSS Inc.) and compost treatments were compared by the least significant difference (LSD) test. A probability level of $\alpha=0.05$ was applied to determine statistical significance.

3. Results

3.1 - Dead plants

The runner bean crop was at plant density 3.3 stems m⁻² (12 stems/3.6 m²), meaning that the number of normal and self-grafted plants with one stem per plant, was double (33000 plants ha⁻¹), the double-stemmed grafted plants (16667 plants ha⁻¹). The number of plants per crop treatment throughout the field experiment was two for the grafted plants and four for the normal and self-grafted plants occupying the same area. Thus, the evaluation of dead stems considered the four stems in observation.

None of the plants in treatments self-grafted cv. Rajado (RR), and cv. Oriente grafted onto rootstock P3 (P3O) died. The first crop treatment where plants started to die 71 days after planting (DAP) was the cv. Oriente grafted onto rootstock P2 (P2O), followed by the same cv. grafted onto rootstock P1 (P1O) and cv. Rajado grafted onto rootstock P2 (P2R) 78 DAP; the cv. Rajado grafted onto rootstock P1 (P1R) and self-grafted cv. Oriente (OO) 82 DAP and, finally, cv. Rajado grafted onto rootstock P3 (P3R) 85 DAP. Only P1O e P2R had more dead stems after the first ones, respectively at 82 e 89 DAP (Fig. 3.1).

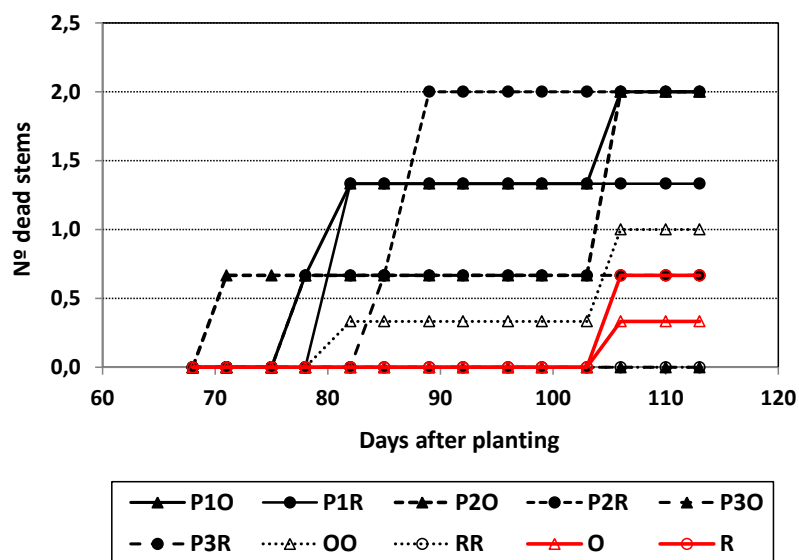


Figure 3.1 - Mean number of dead stems (out of 4) from 68 days after planting, for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.

The final number of dead stems was significantly higher for the rootstocks P2 (2.0 dead stems) compared to the rootstocks P3 (0.3 dead stems) and was similar to the other crop treatments (Fig. 3.2 a). Both cultivars Oriente and Rajado had similar final number of dead stems (Fig. 3.2 b).

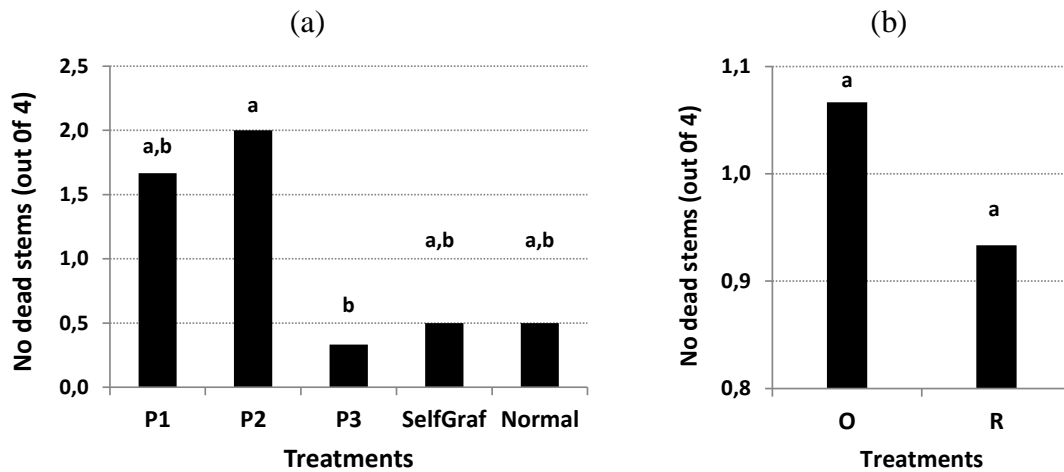


Figure 3.2 - Final number of dead stems for (a) the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants) and for (b) all crop treatments of the cv. Oriente and cv. Rajado. Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

3.2 - Crop development and growth

The first flower appeared earlier in plants grafted onto the P3 rootstock and the ungrafted crop treatments (mean 35.9 DAP) compared to P1 and self-grafted (mean 42.2 DAP) and were similar to P2. Consequently the first pod was observed approximately 6 days after the first flower, earlier for the P3, ungrafted as well as P2 crop treatments (mean 42 DAP), compared to P1 and self-grafted (mean 47.9 DAP), (Fig. 3.3 a). The cv. Oriente presented the first flower three days earlier than cv. Rajado, although the first pod appeared at the same time for both cultivars (mean of 44.4 DAP) (Fig. 3.3 b).

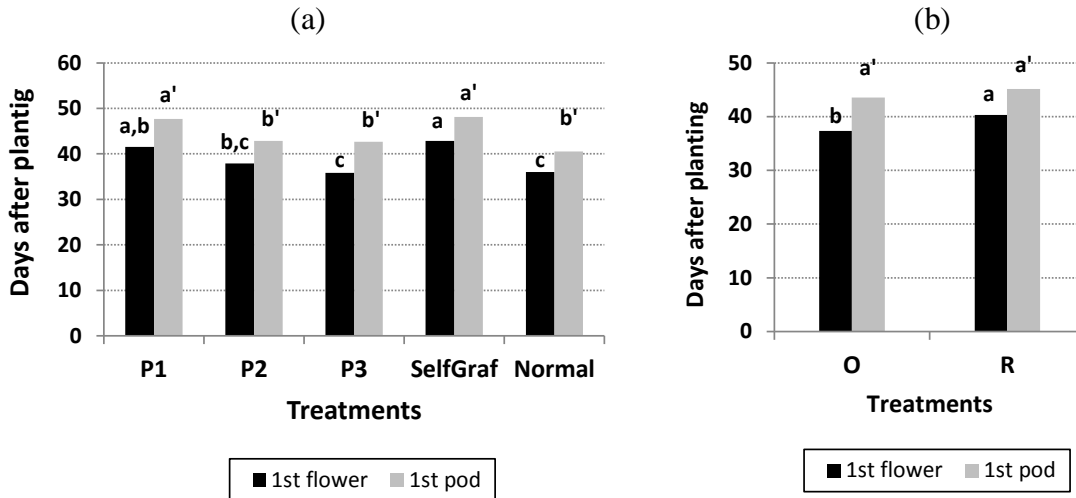


Figure 3.3 - Days after planting of the first flower and first pod appearance for (a) the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants) and for (b) all crop treatments of the cv. Oriente and of the cv. Rajado. Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

The interaction between the rootstock crop treatments (P1, P2, P3, self-grafted and ungrafted) and the two runner bean cultivars (Oriente and Rajado) was not significant for all crop measured parameters.

The accumulated number of pods throughout the growing period, represented in Figure 3.4, showed that the runner bean plants grafted onto rootstock P3 produced more pods than the other crop treatments from 89 DAP onwards. In fact, the total number of pods (m^{-2}) produced was significantly higher for this crop treatment ($p < 0.05$) compared to the others (Fig. 3.5), and the two cultivars produced similar total number of pods (Fig. 3.11 b).

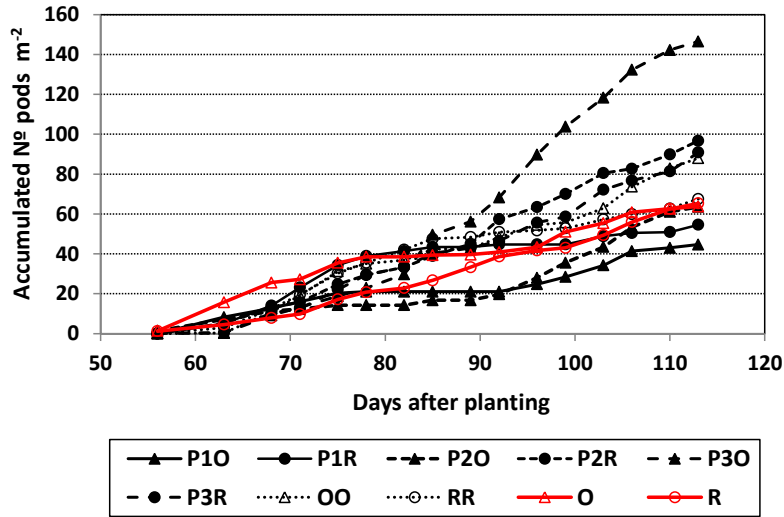


Figure 3.4 - Accumulated number of pods throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.

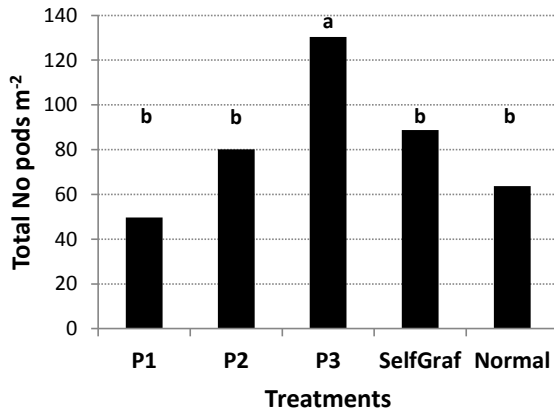


Figure 3.5 - Total number of pods (m⁻²) for the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

The accumulated fresh weight of pods throughout the growing period, showed a similar pattern to the number of pods (Fig. 3.6), and the yield for the grafted plants onto rootstock P3 (1.57 kg m⁻²) was higher compared to the P1, P2 and ungrafted plants, with no significant differences with the self-grafted plants (Fig. 3.7).

Although the interaction between rootstock and cultivar treatments were not significant, the analysis of variance of yield data for each cultivar showed that no significant differences were found between crop treatments for cv. Rajada, although P2 and P3 rootstocks doubled crop yield (1.0 kg m⁻²) compared to the other three crop treatments (mean of 0.5 kg m⁻²) (Fig. 3.8). For the cv. Oriente the rootstock P3 induced to a significant higher yield (2.1 kg m⁻²) compared to

ungrafted and grafted onto rootstocks P1 and P2 plants (mean of 0.8 kg m⁻²), although yield of self-grafted plants were similar to all crop treatments (Fig. 3.8).

Yield of the cv. Oriente (1.2 kg m⁻²) was higher ($p < 0.05$) compared to the cv. Rajado (0.7 kg m⁻²) (Fig. 3.12).

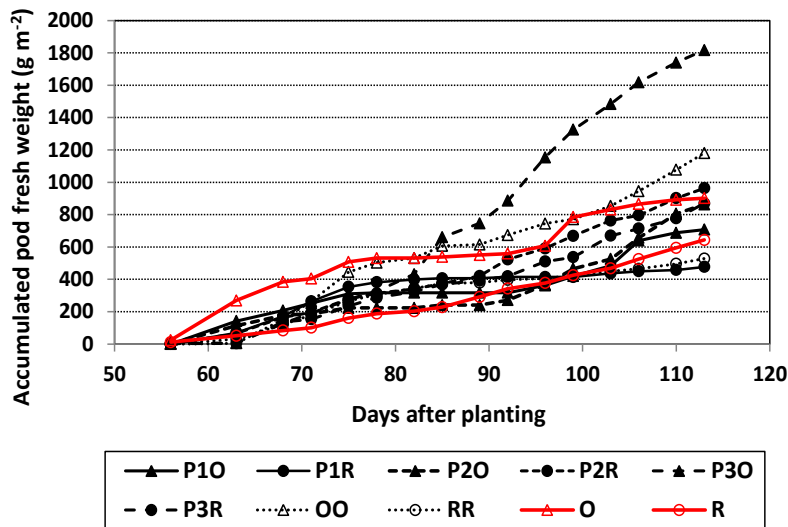


Figure 3.6 - Accumulated pods fresh weight (g m⁻²) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.

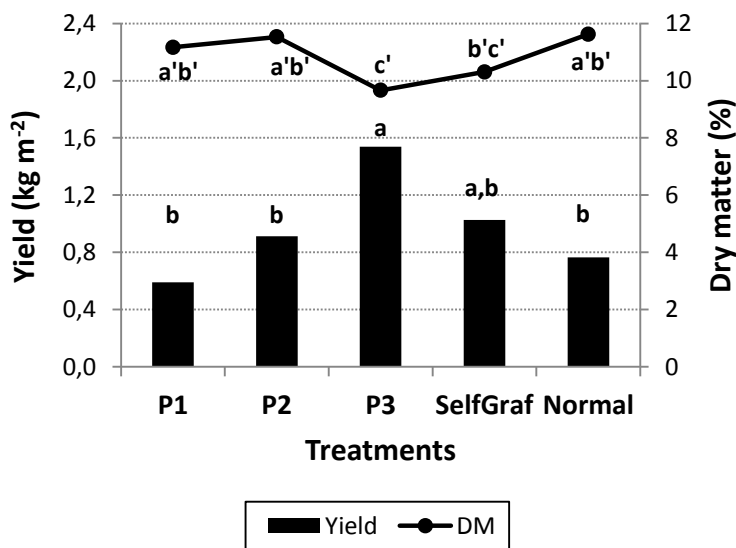


Figure 3.7 - Crop yield (kg m⁻²) and pod dry matter (%) for the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

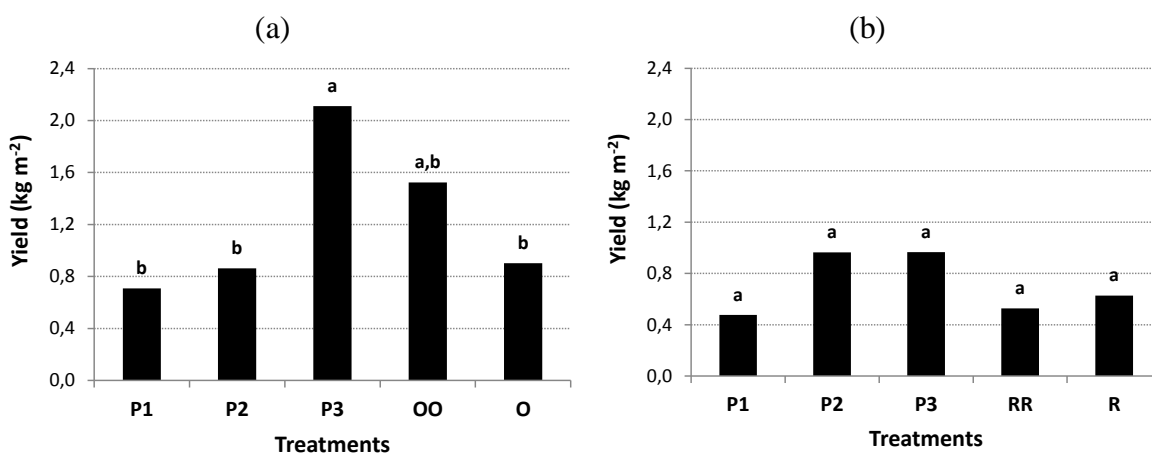


Figure 3.8 - Crop yield (kg m⁻²) for the (a) cv. Oriente and (b) cv. Rajado, grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

From 75 DAP all crops suffered from lack of nutrients, mainly nitrogen, which was applied at 82 DAP (1 June) leading to an increase of plant development and growth, perceived approximately 10 days after (Fig. 3.6). In addition, at about the same period symptoms of the diseases caused by *Fusarium oxysporum* and *Fusarium solani*, were detected, and therefore a fungicide was applied twice, at 84 and 98 DAP. The rootstock P3 seemed to be the one that withstood better these abiotic and biotic stresses, being more tolerant to them.

3.3 - Dry matter content of the pods

Pod dry matter ranged between 9.0% and 13.1% throughout the growing period (Fig. 3.9) and the mean pod dry matter was significantly lower ($p < 0.05$) in the P3 (9.7%) than in the P1, P2 and ungrafted crop treatments (mean 11.4%), with no significant differences compared to the self-grafted treatment (Fig. 3.7).

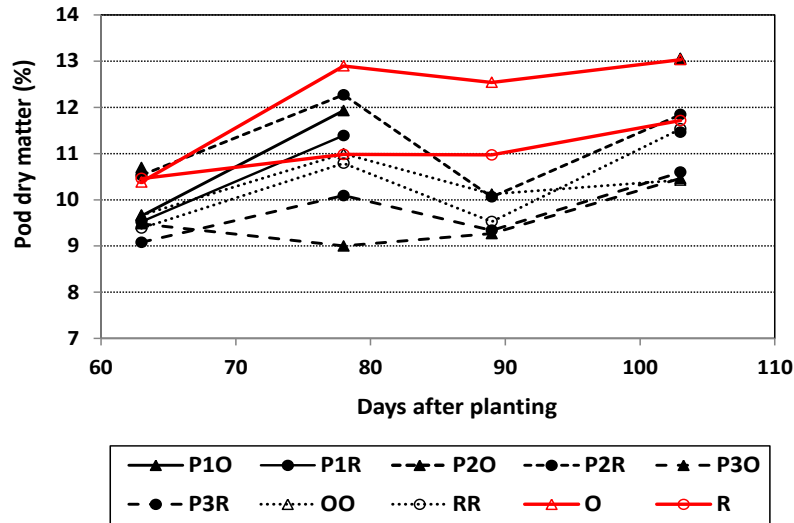


Figure 3.9 - Pod dry matter (%) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.

3.4 - Pod length

The mean pod length throughout the growing period tends to diminish throughout the growing period for both runner bean cultivars (Fig.3.10). The higher pod length was found in the P3 (16.8 cm pod⁻¹), compared to P1, self-grafted and ungrafted crop treatments (mean of 14.9 cm pod⁻¹) and similar to the P2 (16.1 cm pod⁻¹), which was not significantly different compared to the other crop treatments (Fig. 3.11). The mean pod length of cv. Oriente was 17.0 cm pod⁻¹ and was higher ($p < 0.05$) than the mean pod length of cv. Rajado (14.0 cm pod⁻¹) (Fig. 3.12 a).

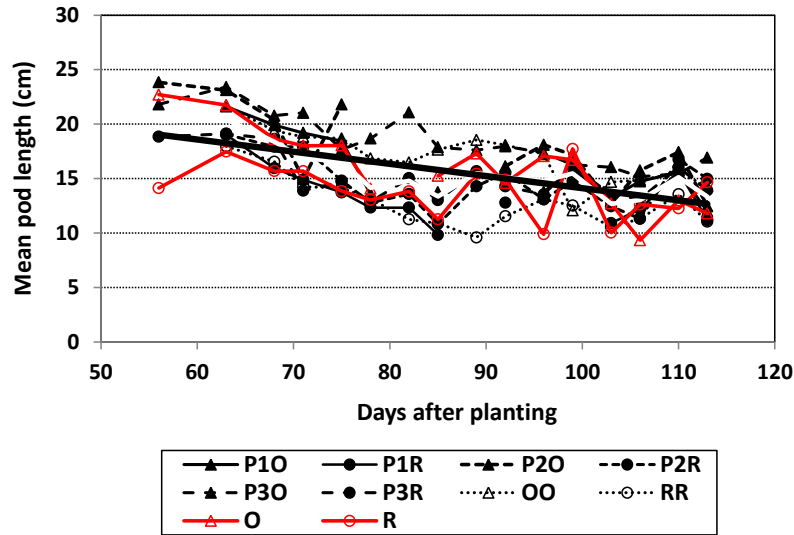


Figure 3.10 - Mean pod length (cm pod⁻¹) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3. The line is the linear model for the overall mean pod length in each harvest.

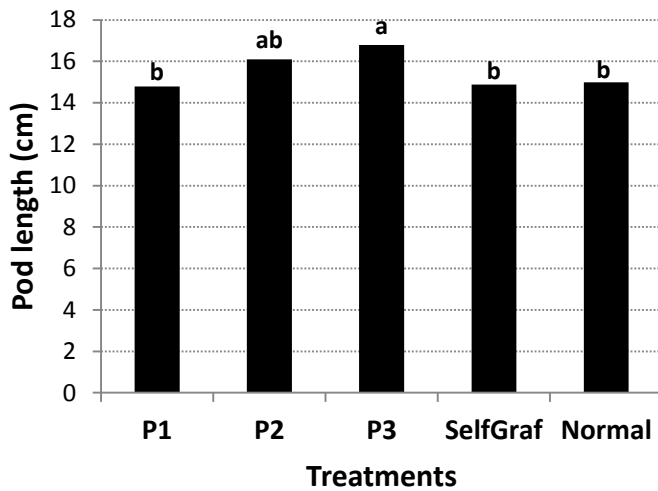


Figure 3.11 - Mean pod length (cm pod⁻¹) for the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants). Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

Yield of the cv. Oriente was higher compared to the cv. Rajado mainly due to the longer pods as the total number of pods was similar (Fig. 3.12).

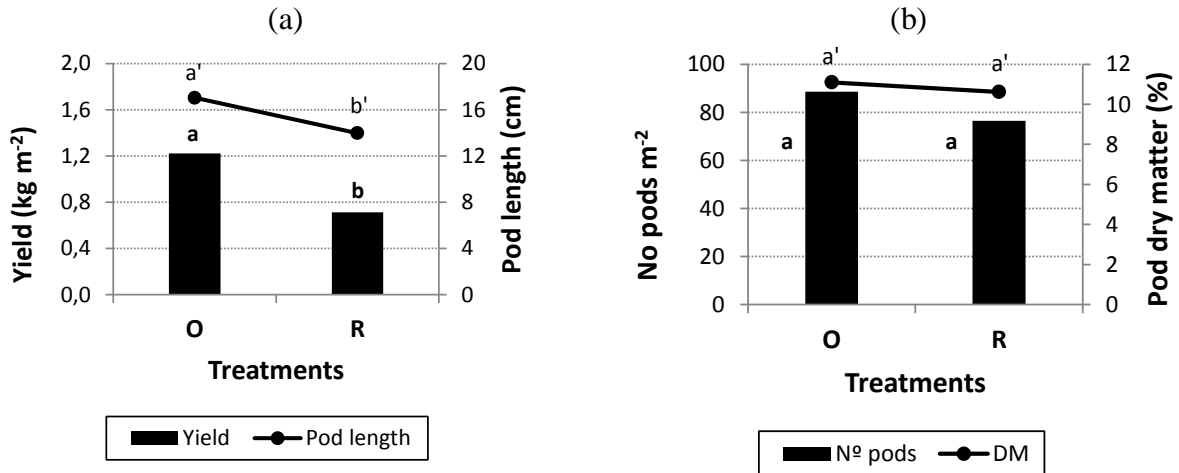


Figure 3.12 - (a) Crop yield (kg m^{-2}) and mean pod length (cm pod^{-1}); (b) total number of pods (m^{-2}) and pod dry matter (%), for all crop treatments of the cv. Oriente and of the cv. Rajado. Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

3.5 - Pod defects

The pod defects considered were classified as minor when pods presented a little twist or bend, and as serious when pods were greatly bent or distorted and when disease symptoms were present. The minor defects were the main problem found in the pods and were present throughout the growing period (Fig. 3.13). The number of pods with serious defects (mean of 10.3 pods m^{-2}) and with disease symptoms (mean of 1.8 pods m^{-2}) were similar for all crop treatments and for the two runner bean cultivars (Fig. 3.14).

The minor defects were higher ($p < 0.05$) in the P3 crop treatment (32.0 pods m^{-2}) compared to the other crop treatments (mean of 23.2 pods m^{-2}) (Fig. 3.14 a), but the percentage of these pods from the total number of pods were 24.6% for the P3 and was 33.9% for the other crop treatments.

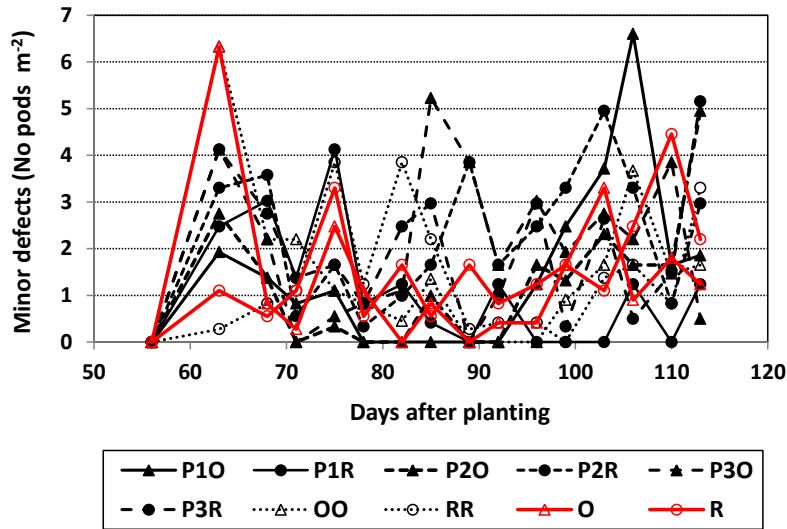


Figure 3.13 - Minor pod defects (No of pods m⁻²) throughout the growing period for the runner bean plants of cv. Oriente and cv. Rajado, ungrafted (O, R), self-grafted (OO, RR) and grafted onto rootstocks P1, P2 and P3.

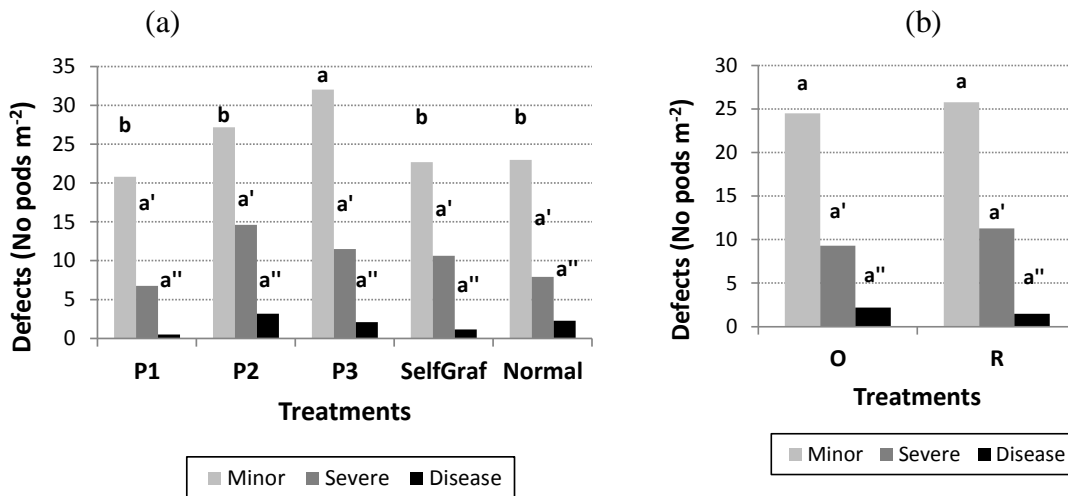


Figure 3.14 - Pod defects (minor, severe and with disease symptoms) (No of pods m⁻²), for (a) the two runner bean cultivars grafted onto rootstocks P1, P2 and P3, self-grafted and ungrafted (normal plants) and for (b) all crop treatments of the cv. Oriente and of the cv. Rajado. Different letters above bars mean significant differences between crop treatments ($p < 0.05$).

4. Discussion and conclusions

4.1 - Crop development and growth

The main objective of the present field experiment was to investigate the effect of three rootstocks for two commercial runner bean cultivars and, for this purpose, the rootstock P3 seemed to perform better than the rootstocks P1 and P2, at least for the cv. Oriente.

The plants from cv. Oriente grafted onto this rootstock P3 did not die and for cv. Rajado the plants grafted onto P3 were the last grafted crop treatment showing dead stems. The crop development was similar between the rootstock P3 and ungrafted plants as well as rootstock P2. The first flower appeared at the same time and earlier when compared to P1 and self-grafted crop treatments, although the fruit set was at the same approximate time.

The nitrogen deficiency due to poor crop management and the presence of soilborne diseases were abiotic and biotic stresses that the rootstock P3 seemed to withstand better than the other rootstocks, at least for cv. Oriente. Probably a more developed and deeper root system of P3 allowed the uptake of more nutrients and a higher tolerance to *Fusarium* spp. could explain the better performance.

Rootstock P3 produced a higher total number of pods and higher yield when compared to the P1, P2 and ungrafted plants, with no significant differences with the self-grafted plants. However, these results were due to the results obtained by cv. Oriente, as yield data analysis for each cultivar showed that no significant differences were found between crop treatments for cv. Rajado, although P2 and P3 crop treatments doubled crop yield (1.0 kg m^{-2}) compared to the other crop treatments (0.5 kg m^{-2}). On the other hand, for cv. Oriente the rootstock P3 induced to a significantly higher yield (2.1 kg m^{-2}) compared to ungrafted and grafted onto rootstocks P1 and P2 plants (mean of 0.8 kg m^{-2}), although yield of self-grafted plants were similar to all crop treatments for this cultivar.

The interaction between the rootstock crop treatments (including the self-grafted and the ungrafted plants) and the two runner bean cultivars (Oriente and Rajado) was not significant for all measured crop parameters, meaning that the effect of grafting was not dependent on the crop cultivars.

Both cultivars, Oriente and Rajado, had similar responses for the final number of dead stems. The crop development was similar between them, as cv. Oriente presented the first flower three days earlier than cv. Rajado, but the first pod appeared approximately at the same time.

The overall mean yield of the cv. Oriente (1.2 kg m^{-2}) was higher ($p < 0.05$) compared to the cv. Rajado (0.7 kg m^{-2}), mainly due to the longer pods as the total number of pods was similar.

4.2 - Crop quality

Pod dry matter followed the yield response; the higher yield corresponded to lower pod dry matter, which can be due to higher rate of pod growth.

The higher pod length was found in the P3 (16.8 cm pod⁻¹), compared to P1, self-grafted and ungrafted crop treatments (mean of 14.9 cm pod⁻¹) and similar to the P2 (16.1 cm pod⁻¹), which was not significantly different compared to the other crop treatments.

The mean pod length is cultivar dependent as it is a characteristic of each cultivar. Oriente has longer pods (mean of 17.0 cm pod⁻¹) than cv. Rajado (14.0 cm pod⁻¹) which is a standard traditional runner bean.

The minor defects were higher in the P3 compared to the other crop treatments but the percentage of these pods from the total number was lower, 24.6% for plants grafted onto this P3 rootstock, compared to the other crop treatments (33.9%). There were no differences between the two cultivars for the number of pods with different defect types.

In conclusion, grafting runner bean seems to be an appropriate strategy to increase crop tolerance to soilborne diseases caused by *Fusarium* spp. and nutrient uptake, mainly for higher yielded cultivars as cv. Oriente. For this cv. the rootstock P3 was recommended, while for cv. Rajado, further investigation should continue to evaluate the effects of rootstocks P2 and P3. Unlike rootstocks 1 (cv. Aintree) and 2 (cv. White Emergo Snowy), originally obtained from Tozerseeds, rootstock 3 (cv. Feijão de 7 anos) is a Portuguese, local, cultivar. It has not been selected, improved or produced *en masse*, but rather handed down between farmers for generations. Therefore, it cannot be considered a stabilized cultivar, and the observed differences between this rootstock and the commercially-available ones must be interpreted in light of this. This cultivar shows great potential for use as a runner bean rootstock in cultivation under the tested conditions. It is likely that, after appropriate selection, levels of stress tolerance and yield will be significantly higher and less variable as those observed in this trial. This is a promising output of the work undertaken, and more research needs to be done to stabilize the cultivar, assess the levels of resistance to soil-borne pathogens in controlled conditions, and evaluate the response of Feijão de 7 anos to various abiotic conditions, including temperature, salinity, water and nutrient stresses, all of which have become serious issues for agricultural systems in the last decades. This will form part of an overall strategy to contribute to the development of a more sustainable, ecologically-sound and socially-responsible agricultural food production.

Bibliography

- Adams, C. R. (1984). *Principles of horiculture*. Oxford: Heinemann Professional Publishing Ltd, 101.
- Bausher, M. G. (2011). Grafting Technique to Eliminate Rootstock. *HortScience* 46(4), 596–598.
- Besri, M. (2005). Current Situation of Tomato Grafting as Alternative to Methyl Bromide for Tomato Production in the Mediterranean Region. *Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions*, (62/1-5). San Diego, CA USA.
- Black, L. L. (May 2003). Production in the Hot-Wet Season. *AVRDC pub #03-551*, 3-6.
- Castagnone-Sereno, P. E., Danchin, G. J. (2014). Parasitic success without sex – the nematode experience. *J. of Evolutionary Biology*, 27, 1323-1333.
- Canwell, M. (Ed.). (2013, November 1). Bean, Snap: Recommendations for Maintaining Postharvest Quality. Retrieved from <http://postharvest.ucdavis.edu/pfvegetable/BeansSnap>
- Charles, L. (2005). Phylogenetic Analysis of *Pasteuria penetrans* by Use of Multiple Genetic Loci. *Journal of bacteriology*, 5700–5708.
- Costa, S. R. (2014). Nematodes Ecology And Vegetable Grafting. Annual Conference - Innovation In Vegetable Grafting For Sustainability, Carcavelos, Portugal, 12.
- Costa S.R., Van der Putten W., Kerry B.R. (2011). Microbial ecology and nematode control in natural ecosystems. In: *Davies K and Spiegel Y Eds. Biological control of plant-parasitic nematodes*. Progress in Biological control: building coherence between microbial ecology and molecular mechanisms. Springer.
- Colla, G., Roupael, Y. (2010). Role of grafting in vegetable crops grown under saline conditions. *Scientia Horticulturae*, 147-155.
- Colla, G. (2012). Grafting cucumber plants enhance tolerance to sodium chloride. *Scientia Horticulturae*, 177-185.
- David, L., Vivian C. (2001). Apomictic, polyphagous root-knotnematodes: exceptionally successful and damaging biotrophic root pathogens. *Annu. Rev. Phytopathol.* 39, 53–77.
- Denisen, E. L. (1979). *Principles of Horticulture*. New York: Macmillan Publishing.
- Dong, H.H., Niu, Y.H., Li, W.J., Zhang, D.M. (2008). Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. *J. Exp. Bot.* 59, 1295–1304.
- Dimitrios, S. (2011). Effects of three commercial rootstocks on mineral nutrition, fruit yield, and nutrient uptake by tomato. *J. Plant Nutr. Soil Sci.*, 154-162.
- Edmond, J. (1975). *Fundamentals of Horticulture*. New York: McGraw-Hill, Inc.

- Elmore, C. L., Stapleton J. J., Bell C. E., DeVay J. E. (1997). *Soil Solarization: A Nonpesticidal Method for Controlling, Diseases, Nematodes, and Weeds*. Oakland: Univ. Calif. Agric. Nat. Res. Publ. 21377.
- EPA. (2015, January 9). Methyl Bromide Alternatives. *Ozone Layer Protection - Regulatory Programs*. Retrieved from <http://www.epa.gov/ozone/mbr/alts.html>
- FLF. (2010). Plantas enxertadas conquistam portuguesas. *Frutas Legumes e Flores*, 110, 40-41.
- Gliessman, S. (2013). Agroecology: growing the roots of resistance. *Agroecology and Sustainable food systems*, 37, 19-31.
- Gowen, S.R. (1997). Chemical control of nematodes: efficiency and side-effects. *Plant Nematode Problems and their Control in the Near East Region*. FAO.
- Gurusamy, V. (2010). Grafting as a tool in common bean breeding. *Can. J. Plant Sci*, 90, 299-304.
- Hagedorn, D.J., Inglis, D.A. (1986). *Handbook of bean diseases*. Cooperative Extension Publications. University of Wisconsin, 16.
- Hills, L. D. (1989). *Month-by-month organic gardening*. Wellingburg: Thorsons publishing group.
- House, L. (1982). *Control of pests and diseases of field vegetables*. Alnwick: Crown copyright.
- Hubert, D. W. (1983). *The scientific principles of crop protection*. London: Edward Arnold Ltd, 13-15.
- Jones, J., Haegeman A., Danchin, G. J., Gaur, H. S., at al. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14(9), 946–961.
- Kerry, B. (1997). Biological control of nematodes: prospects and opportunities. *Plant Nematode Problems and their Control in the Near East Region report*. FAO.
- King, S. R. (2008). Grafting for disease resistance. *HortScience*, 1673-1676.
- Kubota, Ch. (2015, February 3). How to Graft Tomato. Retrieved from http://cals.arizona.edu/grafting/howto/tomatoes/grafting_method
- Lee, J.M., Oda, M. (2003). Grafting of herbaceous vegetable and ornamental crops. *Hortic. Rev.* 28, 61–124.
- Lee, J.M., Kubota, C., Tsao, S.J., Bie, ..., Oda, M.(2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Scientia Horticulturae*, 93-105.
- Mäder, J. A. (2010). Evidence for the effectiveness of the Montreal Protocol to protect the ozone layer. *Atmospheric Chemistry and Physics*, 161-171.
- Mourão, I. (2014). Effect of grafting on runner bean growth, yield and auqlity. *Annual Conference - Innovation In Vegetable Grafting For Sustainability*. Carcavelos, Portugal, p. 30.
- Louws, F. J., Rivard C. L., Kubota C. (2010). Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Scientia Horticulturae*, 127, 127–146.

- Louws, C. L. (2008). Grafting to Manage Soilborne Diseases in Heirloom Tomato Production. *HortScience*, 2104-2111.
- Martins, R.C., Silva, C.L.M. (2004). Green beans (*Phaseolus vulgaris*, L.) quality loss upon thawing. *Journal of Food Engineering*, 65, 37–48.
- Masayuki, O. (1999). Grafting of Vegetables to Improve Greenhouse Production. *Bulletin*. Sakai Osaka, Japan: Osaka Prefecture University.
- McAvoy, R. (2015, February 2). *Grafting Techniques for Greenhouse Tomatoes*. Retrieved from University of Massachusetts Amherst: <http://extension.umass.edu/floriculture/factsheets/grafting-techniques-greenhouse-tomatoes>
- McClure, C. K. (2008). Vegetable Grafting: History, Use, and Current Technology Status in North America. *HortScience*. Retrieved from <http://hortsci.ashspublications.org/content/43/6/1664.short>
- Mullin, B.A., Abawi, G.S. (1991). Contribution of Root and Shoot Tissues of *Phaseolus vulgaris* to *Meloidogyne incognita* Resistance. *Hortscience* 26(12):1503-1504.
- Nicol J. M., Turner S. J., Coyne D. L. , Nijs L., Hockland S., Tahna M. Z. (2011). Current Nematode Threats to World Agriculture. In: Jones et al Eds *Genomics and Molecular Genetics of Plant-Nematode Interactions*. Springer
- Nijs, D. (1984). Rootstock-scion interactions in the cucumber: implications for cultivation and breeding. *Acta Horticulturae* 156, 53-60.
- Nobel Prize: http://www.nobelprize.org/nobel_prizes/peace/laureates/1970/borlaug-facts.html, consulted on the 08th July 2015.
- Nonnecke, I. L. (1989). *Vegetable production*. New York: Van Nostrand Reinhold.
- Oda, M. (1999). *Grafting of Vegetables to Improve Greenhouse Production*. Osaka : Osaka Prefecture University bulletin.
- Oerke, E. C. (2005). Centenary Review. Crop losses to pests. *The Journal of Agricultural Science*, 31-43. Fig. 6
- Pynenburg, G. M. (2011, January 12). Argonomic and economic assessment of intensive pest management of dry bean. *Crop Protection*, 340-348.
- Queensland, T. G. (2015, January 20). Root-knot nematode. Retrieved from Department of Agriculture, Fishery and Forestry: <https://www.daff.qld.gov.au/about-us/the-organisation>
- RHS. (2015, 1 19). Runner beans advice. Retrieved from Royal Horticultural Society: <https://www.rhs.org.uk/advice/profile?PID=667>
- Rodrigues, C. (2010). Plantas enxertadas. *Cultivo horticolas*, 40-41.
- Rodrigues, C. L. (2009). I Colóquio Nacional de Sementes e Viveiros. *Plantas hortícolas enxertadas*, (pp. 80-84). Coimbra.

- Rivero, R. M. (2003). Role of grafting in horticultural plants under stress conditions. *Food, Agriculture & Environment* Vol.1(1), 70-74.
- Rubatzky, V. E. (1997). *World Vegetables: principles, nutrition, and nutritive values*. New York: Chapman & Hall
- Ruiz, J.M., (1997). Leaf-macronutrient content and yield in grafted. *Scientia Horticulturae*, 71 , 227-234 .
- Salehi-Mohammadi, R., Khasi, A., Lee, S.G., Huh, Y.C., Lee, J.M., Delshad, M. (2009). Assessing survival and growth performance of Iranian melon to grafting onto Cucurbita rootstocks. *Korean J. Hortic. Sci. Technol.* 27 (1), 1–6.
- Sasser, J. N. (1985). *Overview of the International Meloidogyne Project 1975–1984*. In *An Advanced Treatise on Meloidogyne*. Raleigh, North Carolina: North Carolina State University Graphics, 271.
- Singh, S. P., Howard, F. S. (2010). Review: Breeding common bean for resistance to insect pests and nematodes. *J. Plant Sci*, 91, 239-250.
- SmartGardenerInc. (2014). Runner bean pests. Retrieved from [www.smartgardener.com: http://www.smartgardener.com/plants/5422-bean-scarlet-runner-white-emergo-runner-bean/pests/771-nematodes](http://www.smartgardener.com/plants/5422-bean-scarlet-runner-white-emergo-runner-bean/pests/771-nematodes)
- Soledad, V.-L., Blanco, M., Cortada, L., Sorribas, J. F. (2013). Resistance of tomato rootstocks to *Meloidogyne arenaria* and *Meloidogyne javanica* under intermittent elevated soil temperatures above 28 °C. *Crop Protection*, 46, 57-62.
- Splittstoesser, W. E. (1990). *Vegetable growing handbook: Organic and traditional methods*. New York: Van Nostrand Reinhold Ltd.
- Stegemann, S. (2012). Horizontal transfer of chloroplast genomes between plant species. *Proceedings of the National Academy of Sciences of the USA*, 2434–2438.
- Sun, Q., Zhao, D., Wang, Ch., Zhao, Y. Design of a Sapling Branch Grafting Robot. (2014). *Journal of Robotics*, Article ID 604746, 9 pages, 2014. doi:10.1155/2014/604746
- Takahashi, K. (1984). Injury by continuous cropping in vegetables: various problems in the cultivation using grafted plants. *Yasaishikenjo Kenkyu Shiryo*, 87-89.
- Taylor, A. L. (1978). *Biology, Identification And Control Of Root-Knot Nematodes*. Raleigh: North Carolina State University Graphics, 28-36.
- Wesemael M. L., Moens, M. (2011). Screening of common bean (*Phaseolus vulgaris*) for resistance against temperate root-knot nematodes (*Meloidogyne spp.*). *Pest Manag Sci*, 68, 702–708.
- Yagiz, Y., Cecilia M., Nunes, N. (2010). Quality Attributes Limiting Snap Bean (*Phaseolus vulgaris* L.) Postharvest Life at Chilling and Non-chilling Temperatures. *HortScience*, 45/8, 1238-1249.

Attachment 1. Statistical analysis of the results

Statistical analysis of the data for the scion cultivar Oriente

Tests of Between-Subjects Effects

* $p > 0.05$ the differences between the treatments are not significant

$p < 0.05$ the treatments are significantly different

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	p*
Rootstock	Dead Stems	10,267	4	2,567	1,158	,397
	Dry Matter	13,516	4	3,379	3,417	,065
	Length of pods	11,431	4	2,858	1,790	,224
	N of Pods	24518,511	4	6129,628	4,346	,037
	Fresh Weight	4424008,573	4	1106002,143	3,343	,069
	Minor defects	788,229	4	197,057	3,850	,050
	Serious defects	115,380	4	28,845	1,590	,267
	Diseases	4,757	4	1,189	,366	,827
	Date of first flower	88,167	4	22,042	2,504	,125
	Date of first pod	35,767	4	8,942	,621	,660
Error	Dead Stems	17,733	8	2,217		
	Dry Matter	7,912	8	,989		
	Length of pods	12,769	8	1,596		
	N of Pods	11284,033	8	1410,504		
	Fresh Weight	2646858,735	8	330857,342		
	Minor defects	409,459	8	51,182		
	Serious defects	145,108	8	18,139		
	Diseases	26,003	8	3,250		
	Date of first flower	70,433	8	8,804		
	Date of first pod	115,133	8	14,392		

a R Squared = ,387 (Adjusted R Squared = -,073)

b R Squared = ,698 (Adjusted R Squared = ,471)

c R Squared = ,495 (Adjusted R Squared = ,117)

d R Squared = ,711 (Adjusted R Squared = ,495)

e R Squared = ,671 (Adjusted R Squared = ,424)

f R Squared = ,690 (Adjusted R Squared = ,457)

g R Squared = ,668 (Adjusted R Squared = ,420)

h R Squared = ,231 (Adjusted R Squared = -,346)

i R Squared = ,557 (Adjusted R Squared = ,224)

j R Squared = ,280 (Adjusted R Squared = -,260)

Pairwise Comparisons

Dependent Variable	(I) PortaEnx	(J) PortaEnx	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)		
						Upper Bound	Lower Bound	
Dead Stems	Normal	P1	-1,667	1,216	,208	-4,470	1,137	
		P2	-1,667	1,216	,208	-4,470	1,137	
		P3	,333	1,216	,791	-2,470	3,137	
		self	-,667	1,216	,598	-3,470	2,137	
	P1	Normal	1,667	1,216	,208	-1,137	4,470	
		P2	,000	1,216	1,000	-2,803	2,803	
		P3	2,000	1,216	,139	-,803	4,803	
		self	1,000	1,216	,435	-1,803	3,803	
	P2	Normal	1,667	1,216	,208	-1,137	4,470	
		P1	,000	1,216	1,000	-2,803	2,803	
		P3	2,000	1,216	,139	-,803	4,803	
		self	1,000	1,216	,435	-1,803	3,803	
	P3	Normal	-,333	1,216	,791	-3,137	2,470	
		P1	-2,000	1,216	,139	-4,803	,803	
		P2	-2,000	1,216	,139	-4,803	,803	
		self	-1,000	1,216	,435	-3,803	1,803	
	self	Normal	,667	1,216	,598	-2,137	3,470	
		P1	-1,000	1,216	,435	-3,803	1,803	
		P2	-1,000	1,216	,435	-3,803	1,803	
		P3	1,000	1,216	,435	-1,803	3,803	
	Dry Matter	Normal	P1	,700	,812	,414	-1,172	2,572
			P2	,200	,812	,812	-1,672	2,072
			P3	2,567(*)	,812	,013	,694	4,439
			self	1,567	,812	,090	-,306	3,439
P1		Normal	-,700	,812	,414	-2,572	1,172	
		P2	-,500	,812	,555	-2,372	1,372	
		P3	1,867	,812	,051	-,006	3,739	
		self	,867	,812	,317	-1,006	2,739	
P2		Normal	-,200	,812	,812	-2,072	1,672	
		P1	,500	,812	,555	-1,372	2,372	
		P3	2,367(*)	,812	,019	,494	4,239	
		self	1,367	,812	,131	-,506	3,239	
P3		Normal	-2,567(*)	,812	,013	-4,439	-,694	
		P1	-1,867	,812	,051	-3,739	,006	
		P2	-2,367(*)	,812	,019	-4,239	-,494	
		self	-1,000	,812	,253	-2,872	,872	
self		Normal	-1,567	,812	,090	-3,439	,306	
		P1	-,867	,812	,317	-2,739	1,006	
		P2	-1,367	,812	,131	-3,239	,506	
		P3	1,000	,812	,253	-,872	2,872	
Length of pods		Normal	P1	-1,100	1,032	,317	-3,479	1,279
			P2	-1,667	1,032	,145	-4,045	,712
			P3	-2,700(*)	1,032	,031	-5,079	-,321
			self	-1,300	1,032	,243	-3,679	1,079
	P1	Normal	1,100	1,032	,317	-1,279	3,479	

N of Pods	P2	P2	-,567	1,032	,598	-2,945	1,812
		P3	-1,600	1,032	,159	-3,979	,779
		self	-,200	1,032	,851	-2,579	2,179
	P2	Normal	1,667	1,032	,145	-,712	4,045
		P1	,567	1,032	,598	-1,812	2,945
		P3	-1,033	1,032	,346	-3,412	1,345
	P3	self	,367	1,032	,731	-2,012	2,745
		Normal	2,700(*)	1,032	,031	,321	5,079
		P1	1,600	1,032	,159	-,779	3,979
	self	P2	1,033	1,032	,346	-1,345	3,412
		self	1,400	1,032	,212	-,979	3,779
		Normal	1,300	1,032	,243	-1,079	3,679
	Normal	P1	,200	1,032	,851	-2,179	2,579
		P2	-,367	1,032	,731	-2,745	2,012
		P3	-1,400	1,032	,212	-3,779	,979
	P1	P1	11,333	30,665	,721	-59,380	82,047
		P2	5,300	30,665	,867	-65,413	76,013
		P3	-96,233(*)	30,665	,014	-166,947	-25,520
	P2	self	-40,500	30,665	,223	-111,213	30,213
		Normal	-11,333	30,665	,721	-82,047	59,380
		P2	-6,033	30,665	,849	-76,747	64,680
	P3	P3	-107,567(*)	30,665	,008	-178,280	-36,853
		self	-51,833	30,665	,129	-122,547	18,880
		Normal	-5,300	30,665	,867	-76,013	65,413
	P2	P1	6,033	30,665	,849	-64,680	76,747
		P3	-101,533(*)	30,665	,011	-172,247	-30,820
		self	-45,800	30,665	,174	-116,513	24,913
	P3	Normal	96,233(*)	30,665	,014	25,520	166,947
		P1	107,567(*)	30,665	,008	36,853	178,280
		P2	101,533(*)	30,665	,011	30,820	172,247
self	self	55,733	30,665	,107	-14,980	126,447	
	Normal	40,500	30,665	,223	-30,213	111,213	
	P1	51,833	30,665	,129	-18,880	122,547	
P2	P2	45,800	30,665	,174	-24,913	116,513	
	P3	-55,733	30,665	,107	-126,447	14,980	

Fresh Weight ORIENTE	Normal	P1	124,033	469,650	,798	-958,983	1207,049	
		P2	97,333	469,650	,841	-985,683	1180,349	
		P3	-1275,200(*)	469,650	,026	-2358,216	-192,184	
	P1	self	-619,333	469,650	,224	-1702,349	463,683	
		Normal	-124,033	469,650	,798	-1207,049	958,983	
		P2	-26,700	469,650	,956	-1109,716	1056,316	
	P2	P3	-1399,233(*)	469,650	,018	-2482,249	-316,217	
		self	-743,367	469,650	,152	-1826,383	339,649	
		Normal	-97,333	469,650	,841	-1180,349	985,683	
	P3	P1	26,700	469,650	,956	-1056,316	1109,716	
		P3	-1372,533(*)	469,650	,019	-2455,549	-289,517	
		self	-716,667	469,650	,166	-1799,683	366,349	
	self	Normal	1275,200(*)	469,650	,026	192,184	2358,216	
		P1	1399,233(*)	469,650	,018	316,217	2482,249	
		P2	1372,533(*)	469,650	,019	289,517	2455,549	
	Minor defects	Normal	self	655,867	469,650	,200	-427,149	1738,883
			Normal	619,333	469,650	,224	-463,683	1702,349
			P1	743,367	469,650	,152	-339,649	1826,383
			P2	716,667	469,650	,166	-366,349	1799,683
			P3	-655,867	469,650	,200	-1738,883	427,149
P1	Normal	P1	5,533	5,841	,371	-7,937	19,004	
		P2	3,667	5,841	,548	-9,804	17,137	
		P3	-15,000(*)	5,841	,033	-28,470	-1,530	
	P1	self	-3,067	5,841	,614	-16,537	10,404	
		Normal	-5,533	5,841	,371	-19,004	7,937	
		P2	-1,867	5,841	,757	-15,337	11,604	
	P2	P3	-20,533(*)	5,841	,008	-34,004	-7,063	
		self	-8,600	5,841	,179	-22,070	4,870	
		Normal	-3,667	5,841	,548	-17,137	9,804	
	P3	P1	1,867	5,841	,757	-11,604	15,337	
		P3	-18,667(*)	5,841	,013	-32,137	-5,196	
		self	-6,733	5,841	,282	-20,204	6,737	
	self	Normal	15,000(*)	5,841	,033	1,530	28,470	
		P1	20,533(*)	5,841	,008	7,063	34,004	
		P2	18,667(*)	5,841	,013	5,196	32,137	
Serious defects	Normal	self	11,933	5,841	,075	-1,537	25,404	
		Normal	3,067	5,841	,614	-10,404	16,537	
		P1	8,600	5,841	,179	-4,870	22,070	
		P2	6,733	5,841	,282	-6,737	20,204	
		P3	-11,933	5,841	,075	-25,404	1,537	
P1	Normal	P1	,567	3,477	,875	-7,452	8,586	
		P2	-2,833	3,477	,439	-10,852	5,186	
		P3	-6,933	3,477	,081	-14,952	1,086	
P2	self	-4,300	3,477	,251	-12,319	3,719		
	Normal	-,567	3,477	,875	-8,586	7,452		
	P2	-3,400	3,477	,357	-11,419	4,619		
P2	Normal	P3	-7,500	3,477	,063	-15,519	,519	
		self	-4,867	3,477	,199	-12,886	3,152	
			2,833	3,477	,439	-5,186	10,852	

Diseases	P3	P1	3,400	3,477	,357	-4,619	11,419
		P3	-4,100	3,477	,272	-12,119	3,919
		self	-1,467	3,477	,684	-9,486	6,552
	self	Normal	6,933	3,477	,081	-1,086	14,952
		P1	7,500	3,477	,063	-,519	15,519
		P2	4,100	3,477	,272	-3,919	12,119
	Normal	self	2,633	3,477	,471	-5,386	10,652
		Normal	4,300	3,477	,251	-3,719	12,319
		P1	4,867	3,477	,199	-3,152	12,886
	P1	P2	1,467	3,477	,684	-6,552	9,486
		P3	-2,633	3,477	,471	-10,652	5,386
		self	,567	1,472	,710	-2,828	3,961
	P2	P2	-,967	1,472	,530	-4,361	2,428
		P3	-,833	1,472	,587	-4,228	2,561
		self	-,167	1,472	,913	-3,561	3,228
	P3	Normal	-,567	1,472	,710	-3,961	2,828
		P2	-1,533	1,472	,328	-4,928	1,861
		P3	-1,400	1,472	,369	-4,795	1,995
	P2	self	-,733	1,472	,632	-4,128	2,661
		Normal	,967	1,472	,530	-2,428	4,361
		P1	1,533	1,472	,328	-1,861	4,928
	P3	P3	,133	1,472	,930	-3,261	3,528
		self	,800	1,472	,602	-2,595	4,195
		Normal	,833	1,472	,587	-2,561	4,228
self	P1	1,400	1,472	,369	-1,995	4,795	
	P2	-,133	1,472	,930	-3,528	3,261	
	self	,667	1,472	,663	-2,728	4,061	
Date of first flower	Normal	,167	1,472	,913	-3,228	3,561	
	P1	,733	1,472	,632	-2,661	4,128	
	P2	-,800	1,472	,602	-4,195	2,595	
P1	P3	-,667	1,472	,663	-4,061	2,728	
	Normal	P1	-3,667	2,423	,169	-9,253	1,920
	P2	,500	2,423	,842	-5,087	6,087	
P2	P3	2,167	2,423	,397	-3,420	7,753	
	self	-4,000	2,423	,137	-9,587	1,587	
	Normal	3,667	2,423	,169	-1,920	9,253	
P3	P2	4,167	2,423	,124	-1,420	9,753	
	P3	5,833(*)	2,423	,043	,247	11,420	
	self	-,333	2,423	,894	-5,920	5,253	
self	Normal	-,500	2,423	,842	-6,087	5,087	
	P1	-4,167	2,423	,124	-9,753	1,420	
	P3	1,667	2,423	,511	-3,920	7,253	
P3	self	-4,500	2,423	,100	-10,087	1,087	
	Normal	-2,167	2,423	,397	-7,753	3,420	
	P1	-5,833(*)	2,423	,043	-11,420	-,247	
self	P2	-1,667	2,423	,511	-7,253	3,920	
	self	-6,167(*)	2,423	,034	-11,753	-,580	
	Normal	4,000	2,423	,137	-1,587	9,587	
	P1	,333	2,423	,894	-5,253	5,920	
	P2	4,500	2,423	,100	-1,087	10,087	
	P3	6,167(*)	2,423	,034	,580	11,753	

Date of first pod	Normal	P1	-3,500	3,097	,291	-10,643	3,643
		P2	,333	3,097	,917	-6,809	7,476
		P3	,500	3,097	,876	-6,643	7,643
		self	-1,833	3,097	,570	-8,976	5,309
	P1	Normal	3,500	3,097	,291	-3,643	10,643
		P2	3,833	3,097	,251	-3,309	10,976
		P3	4,000	3,097	,233	-3,143	11,143
		self	1,667	3,097	,605	-5,476	8,809
	P2	Normal	-,333	3,097	,917	-7,476	6,809
		P1	-3,833	3,097	,251	-10,976	3,309
		P3	,167	3,097	,958	-6,976	7,309
		self	-2,167	3,097	,504	-9,309	4,976
	P3	Normal	-,500	3,097	,876	-7,643	6,643
		P1	-4,000	3,097	,233	-11,143	3,143
		P2	-,167	3,097	,958	-7,309	6,976
		self	-2,333	3,097	,473	-9,476	4,809
	self	Normal	1,833	3,097	,570	-5,309	8,976
		P1	-1,667	3,097	,605	-8,809	5,476
		P2	2,167	3,097	,504	-4,976	9,309
		P3	2,333	3,097	,473	-4,809	9,476

Based on estimated marginal means

* The mean difference is significant at the ,05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Statistical analysis of the data for the scion cultivar Rajado

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Rootstock	Dead Stems	6,933	4	1,733	1,300	,348
	Dry Matter	4,956	4	1,239	1,406	,315
	Length of pods	8,980	4	2,245	2,235	,155
	N of Pods	4962,523	4	1240,631	1,440	,306
	Fresh Weight	527220,727	4	131805,182	1,806	,221
	Minor defects	533,963	4	133,491	1,733	,235
	Serious defects	48,809	4	12,202	,251	,901
	Diseases	11,663	4	2,916	,772	,573
	Date of first flower	186,067	4	46,517	3,307	,070
	Date of first pod	336,900	4	84,225	8,497	,006
Scion cv	Dead Stems	3,333	2	1,667	1,250	,337
	Dry Matter	3,892	2	1,946	2,209	,172
	Length of pods	2,937	2	1,469	1,462	,288
	N of Pods	2750,757	2	1375,379	1,597	,261
	Fresh Weight	271886,405	2	135943,203	1,863	,217
	Minor defects	37,296	2	18,648	,242	,791
	Serious defects	108,801	2	54,401	1,118	,373
	Diseases	,585	2	,293	,077	,926
	Date of first flower	22,300	2	11,150	,793	,485
	Date of first pod	20,033	2	10,017	1,011	,406
Error	Dead Stems	10,667	8	1,333		
	Dry Matter	7,048	8	,881		
	Length of pods	8,036	8	1,005		
	N of Pods	6891,209	8	861,401		
	Fresh Weight	583883,161	8	72985,395		
	Minor defects	616,237	8	77,030		
	Serious defects	389,359	8	48,670		
	Diseases	30,221	8	3,778		
	Date of first flower	112,533	8	14,067		
	Date of first pod	79,300	8	9,913		

a R Squared = ,490 (Adjusted R Squared = ,108)

b R Squared = ,557 (Adjusted R Squared = ,224)

c R Squared = ,597 (Adjusted R Squared = ,295)

d R Squared = ,528 (Adjusted R Squared = ,174)

e R Squared = ,578 (Adjusted R Squared = ,261)

f R Squared = ,481 (Adjusted R Squared = ,092)

g R Squared = ,288 (Adjusted R Squared = -,246)

h R Squared = ,288 (Adjusted R Squared = -,245)

i R Squared = ,649 (Adjusted R Squared = ,386)

j R Squared = ,818 (Adjusted R Squared = ,682)

Pairwise Comparisons

Dependent Variable	(I) PortaEnx	(J) PortaEnx	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)		
						Upper Bound	Lower Bound	
Dead Stems	Normal	P1	-,667	,943	,500	-2,841	1,507	
		P2	-1,333	,943	,195	-3,507	,841	
		P3	1,11E-016	,943	1,000	-2,174	2,174	
		self	,667	,943	,500	-1,507	2,841	
	P1	Normal	,667	,943	,500	-1,507	2,841	
		P2	-,667	,943	,500	-2,841	1,507	
		P3	,667	,943	,500	-1,507	2,841	
		self	1,333	,943	,195	-,841	3,507	
	P2	Normal	1,333	,943	,195	-,841	3,507	
		P1	,667	,943	,500	-1,507	2,841	
		P3	1,333	,943	,195	-,841	3,507	
		self	2,000	,943	,067	-,174	4,174	
	P3	Normal	-1,11E-016	,943	1,000	-2,174	2,174	
		P1	-,667	,943	,500	-2,841	1,507	
		P2	-1,333	,943	,195	-3,507	,841	
		self	,667	,943	,500	-1,507	2,841	
	self	Normal	-,667	,943	,500	-2,841	1,507	
		P1	-1,333	,943	,195	-3,507	,841	
		P2	-2,000	,943	,067	-4,174	,174	
		P3	-,667	,943	,500	-2,841	1,507	
	Dry Matter	Normal	P1	,233	,766	,769	-1,534	2,001
			P2	-,600	,766	,456	-2,367	1,167
			P3	1,100	,766	,189	-,667	2,867
			self	,633	,766	,433	-1,134	2,401
P1		Normal	-,233	,766	,769	-2,001	1,534	
		P2	-,833	,766	,309	-2,601	,934	
		P3	,867	,766	,291	-,901	2,634	
		self	,400	,766	,616	-1,367	2,167	
P2		Normal	,600	,766	,456	-1,167	2,367	
		P1	,833	,766	,309	-,934	2,601	
		P3	1,700	,766	,057	-,067	3,467	
		self	1,233	,766	,146	-,534	3,001	
P3		Normal	-1,100	,766	,189	-2,867	,667	
		P1	-,867	,766	,291	-2,634	,901	
		P2	-1,700	,766	,057	-3,467	,067	
		self	-,467	,766	,559	-2,234	1,301	
self		Normal	-,633	,766	,433	-2,401	1,134	
		P1	-,400	,766	,616	-2,167	1,367	
		P2	-1,233	,766	,146	-3,001	,534	
		P3	,467	,766	,559	-1,301	2,234	
Length		Normal	P1	,167	,818	,844	-1,720	2,054
			P2	-,933	,818	,287	-2,820	,954
			P3	-1,433	,818	,118	-3,320	,454
			self	,700	,818	,417	-1,187	2,587
	P1	Normal	-,167	,818	,844	-2,054	1,720	

N of Pods	P2	P2	-1,100	,818	,216	-2,987	,787
		P3	-1,600	,818	,086	-3,487	,287
		self	,533	,818	,533	-1,354	2,420
	P2	Normal	,933	,818	,287	-,954	2,820
		P1	1,100	,818	,216	-,787	2,987
		P3	-,500	,818	,558	-2,387	1,387
	P3	self	1,633	,818	,081	-,254	3,520
		Normal	1,433	,818	,118	-,454	3,320
		P1	1,600	,818	,086	-,287	3,487
	self	P2	,500	,818	,558	-1,387	2,387
		self	2,133(*)	,818	,031	,246	4,020
		Normal	-,700	,818	,417	-2,587	1,187
	Normal	P1	-,533	,818	,533	-2,420	1,354
		P2	-1,633	,818	,081	-3,520	,254
		P3	-2,133(*)	,818	,031	-4,020	-,246
	P1	P1	-1,367	23,964	,956	-56,627	53,894
		P2	-29,333	23,964	,256	-84,594	25,927
		P3	-47,600	23,964	,082	-102,861	7,661
	P2	self	-11,000	23,964	,658	-66,261	44,261
		Normal	1,367	23,964	,956	-53,894	56,627
		P2	-27,967	23,964	,277	-83,227	27,294
	P3	P3	-46,233	23,964	,090	-101,494	9,027
		self	-9,633	23,964	,698	-64,894	45,627
		Normal	29,333	23,964	,256	-25,927	84,594
	P1	P1	27,967	23,964	,277	-27,294	83,227
		P3	-18,267	23,964	,468	-73,527	36,994
		self	18,333	23,964	,466	-36,927	73,594
	P2	Normal	47,600	23,964	,082	-7,661	102,861
		P1	46,233	23,964	,090	-9,027	101,494
		P2	18,267	23,964	,468	-36,994	73,527
self	self	36,600	23,964	,165	-18,661	91,861	
	Normal	11,000	23,964	,658	-44,261	66,261	
	P1	9,633	23,964	,698	-45,627	64,894	
P3	P2	-18,333	23,964	,466	-73,594	36,927	
	P3	-36,600	23,964	,165	-91,861	18,661	

Fresh Weight RAJADO	Normal	P1	-72,100	220,583	,752	-580,766	436,566
		P2	-307,000	220,583	,201	-815,666	201,666
		P3	-492,900	220,583	,056	-1001,566	15,766
		self	-46,833	220,583	,837	-555,499	461,832
	P1	Normal	72,100	220,583	,752	-436,566	580,766
		P2	-234,900	220,583	,318	-743,566	273,766
		P3	-420,800	220,583	,093	-929,466	87,866
		self	25,267	220,583	,912	-483,399	533,932
	P2	Normal	307,000	220,583	,201	-201,666	815,666
		P1	234,900	220,583	,318	-273,766	743,566
		P3	-185,900	220,583	,424	-694,566	322,766
		self	260,167	220,583	,272	-248,499	768,832
	P3	Normal	492,900	220,583	,056	-15,766	1001,566
		P1	420,800	220,583	,093	-87,866	929,466
		P2	185,900	220,583	,424	-322,766	694,566
		self	446,067	220,583	,078	-62,599	954,732
	self	Normal	46,833	220,583	,837	-461,832	555,499
		P1	-25,267	220,583	,912	-533,932	483,399
		P2	-260,167	220,583	,272	-768,832	248,499
		P3	-446,067	220,583	,078	-954,732	62,599
Minor defects	Normal	P1	-933	7,166	,900	-17,458	15,592
		P2	-10,867	7,166	,168	-27,392	5,658
		P3	-15,167	7,166	,067	-31,692	1,358
		self	-3,067	7,166	,680	-19,592	13,458
	P1	Normal	,933	7,166	,900	-15,592	17,458
		P2	-9,933	7,166	,203	-26,458	6,592
		P3	-14,233	7,166	,082	-30,758	2,292
		self	-2,133	7,166	,774	-18,658	14,392
	P2	Normal	10,867	7,166	,168	-5,658	27,392
		P1	9,933	7,166	,203	-6,592	26,458
		P3	-4,300	7,166	,565	-20,825	12,225
		self	7,800	7,166	,308	-8,725	24,325
	P3	Normal	15,167	7,166	,067	-1,358	31,692
		P1	14,233	7,166	,082	-2,292	30,758
		P2	4,300	7,166	,565	-12,225	20,825
		self	12,100	7,166	,130	-4,425	28,625
	self	Normal	3,067	7,166	,680	-13,458	19,592
		P1	2,133	7,166	,774	-14,392	18,658
		P2	-7,800	7,166	,308	-24,325	8,725
		P3	-12,100	7,166	,130	-28,625	4,425
Serious defects	Normal	P1	,133	5,696	,982	-13,002	13,269
		P2	-4,500	5,696	,452	-17,635	8,635
		P3	-1,433	5,696	,808	-14,569	11,702
		self	,267	5,696	,964	-12,869	13,402
	P1	Normal	-,133	5,696	,982	-13,269	13,002
		P2	-4,633	5,696	,440	-17,769	8,502
		P3	-1,567	5,696	,790	-14,702	11,569
		self	,133	5,696	,982	-13,002	13,269
	P2	Normal	4,500	5,696	,452	-8,635	17,635

Diseases	P3	P1	4,633	5,696	,440	-8,502	17,769
		P3	3,067	5,696	,605	-10,069	16,202
		self	4,767	5,696	,427	-8,369	17,902
	self	Normal	1,433	5,696	,808	-11,702	14,569
		P1	1,567	5,696	,790	-11,569	14,702
		P2	-3,067	5,696	,605	-16,202	10,069
	Normal	self	1,700	5,696	,773	-11,435	14,835
		Normal	-,267	5,696	,964	-13,402	12,869
		P1	-,133	5,696	,982	-13,269	13,002
	P1	P2	-4,767	5,696	,427	-17,902	8,369
		P3	-1,700	5,696	,773	-14,835	11,435
		P1	2,533	1,587	,149	-1,126	6,193
		P2	1,433	1,587	,393	-2,226	5,093
		P3	1,333	1,587	,425	-2,326	4,993
		self	2,233	1,587	,197	-1,426	5,893
		Normal	-2,533	1,587	,149	-6,193	1,126
		P2	-1,100	1,587	,508	-4,760	2,560
		P3	-1,200	1,587	,471	-4,860	2,460
	P2	self	-,300	1,587	,855	-3,960	3,360
		Normal	-1,433	1,587	,393	-5,093	2,226
		P1	1,100	1,587	,508	-2,560	4,760
	P3	P3	-,100	1,587	,951	-3,760	3,560
		self	,800	1,587	,628	-2,860	4,460
		Normal	-1,333	1,587	,425	-4,993	2,326
self	P1	1,200	1,587	,471	-2,460	4,860	
	P2	,100	1,587	,951	-3,560	3,760	
	self	,900	1,587	,586	-2,760	4,560	
	Normal	-2,233	1,587	,197	-5,893	1,426	
	P1	,300	1,587	,855	-3,360	3,960	
	P2	-,800	1,587	,628	-4,460	2,860	
	P3	-,900	1,587	,586	-4,560	2,760	
	Normal	P1	-7,333(*)	3,062	,044	-14,395	-,272
	Date of first flower	P1	P2	-4,333	3,062	,195	-11,395
P3			-1,833	3,062	,566	-8,895	5,228
self			-9,667(*)	3,062	,013	-16,728	-2,605
P2		Normal	7,333(*)	3,062	,044	,272	14,395
		P2	3,000	3,062	,356	-4,062	10,062
		P3	5,500	3,062	,110	-1,562	12,562
P3		self	-2,333	3,062	,468	-9,395	4,728
		Normal	4,333	3,062	,195	-2,728	11,395
		P1	-3,000	3,062	,356	-10,062	4,062
self	P2	P3	2,500	3,062	,438	-4,562	9,562
		self	-5,333	3,062	,120	-12,395	1,728
		Normal	1,833	3,062	,566	-5,228	8,895
	P3	P1	-5,500	3,062	,110	-12,562	1,562
		P2	-2,500	3,062	,438	-9,562	4,562
		self	-7,833(*)	3,062	,034	-14,895	-,772
	Normal	Normal	9,667(*)	3,062	,013	2,605	16,728
		P1	2,333	3,062	,468	-4,728	9,395
		P2	5,333	3,062	,120	-1,728	12,395
		P3	7,833(*)	3,062	,034	,772	14,895

Date of first pod	Normal	P1	-10,833(*)	2,571	,003	-16,761	-4,905
		P2	-5,000	2,571	,088	-10,928	,928
		P3	-4,833	2,571	,097	-10,761	1,095
		self	-13,333(*)	2,571	,001	-19,261	-7,405
	P1	Normal	10,833(*)	2,571	,003	4,905	16,761
		P2	5,833	2,571	,053	-,095	11,761
		P3	6,000(*)	2,571	,048	,072	11,928
		self	-2,500	2,571	,359	-8,428	3,428
	P2	Normal	5,000	2,571	,088	-,928	10,928
		P1	-5,833	2,571	,053	-11,761	,095
		P3	,167	2,571	,950	-5,761	6,095
		self	-8,333(*)	2,571	,012	-14,261	-2,405
	P3	Normal	4,833	2,571	,097	-1,095	10,761
		P1	-6,000(*)	2,571	,048	-11,928	-,072
		P2	-,167	2,571	,950	-6,095	5,761
		self	-8,500(*)	2,571	,011	-14,428	-2,572
	self	Normal	13,333(*)	2,571	,001	7,405	19,261
		P1	2,500	2,571	,359	-3,428	8,428
		P2	8,333(*)	2,571	,012	2,405	14,261
		P3	8,500(*)	2,571	,011	2,572	14,428

Based on estimated marginal means

* The mean difference is significant at the ,05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Statistical analysis of the data for scion cultivars Rajado and Oriente combined

Tests of Between-Subjects Effects

* $p > 0.05$ the differences between the treatments are not significant
 $p \leq 0.05$ the treatments are significantly different

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	p *
Rootstock	Dead Stems	14,333	4	3,583	2,234	,106
	Dry Matter	16,685	4	4,171	4,798	,008
	Length of pods	17,112	4	4,278	3,247	,036
	N of Pods	22871,312	4	5717,828	4,357	,012
	Fresh Weight	3409380,030	4	852345,008	3,647	,024
	Minor defects	1077,005	4	269,251	4,139	,015
	Serious defects	102,125	4	25,531	,614	,658
	Diseases	10,238	4	2,560	,808	,536
	Date of first flower	245,867	4	61,467	5,720	,004
	Date of first pod	269,367	4	67,342	5,803	,004
Scion Cv	Dead Stems	,133	1	,133	,083	,776
	Dry Matter	,833	1	,833	,959	,341
	Length of pods	77,763	1	77,763	59,017	,000
	N of Pods	1633,932	1	1633,932	1,245	,279
	Fresh Weight	1952790,533	1	1952790,533	8,356	,010
	Minor defects	43,200	1	43,200	,664	,426
	Serious defects	22,707	1	22,707	,546	,469
	Diseases	3,201	1	3,201	1,010	,328
	Date of first flower	66,008	1	66,008	6,142	,023
	Date of first pod	18,408	1	18,408	1,586	,224
Rootstock * Cv interaction	Dead Stems	2,867	4	,717	,447	,773
	Dry Matter	1,787	4	,447	,514	,727
	Length of pods	3,299	4	,825	,626	,650
	N of Pods	6609,721	4	1652,430	1,259	,322
	Fresh Weight	1541849,270	4	385462,318	1,649	,205
	Minor defects	245,187	4	61,297	,942	,462
	Serious defects	62,065	4	15,516	,373	,825
	Diseases	6,182	4	1,546	,488	,745
	Date of first flower	28,367	4	7,092	,660	,628
	Date of first pod	103,300	4	25,825	2,225	,107
Error	Dead Stems	28,867	18	1,604		
	Dry Matter	15,649	18	,869		
	Length of pods	23,717	18	1,318		
	N of Pods	23621,745	18	1312,319		
	Fresh Weight	4206719,321	18	233706,629		
	Minor defects	1170,862	18	65,048		
	Serious defects	748,153	18	41,564		
	Diseases	57,033	18	3,168		
	Date of first flower	193,433	18	10,746		
	Date of first pod	208,900	18	11,606		

Pairwise Comparisons

(*) the treatments are significantly different

Dependent Variable	(I) PortaEnx	(J) PortaEnx	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Upper Bound	Lower Bound
Dead Stems	Normal	P1	-1,167	,731	,128	-2,703	,369
		P2	-1,500	,731	,055	-3,036	,036
		P3	,167	,731	,822	-1,369	1,703
		self	3,33E-016	,731	1,000	-1,536	1,536
	P1	Normal	1,167	,731	,128	-,369	2,703
		P2	-,333	,731	,654	-1,869	1,203
		P3	1,333	,731	,085	-,203	2,869
		self	1,167	,731	,128	-,369	2,703
	P2	Normal	1,500	,731	,055	-,036	3,036
		P1	,333	,731	,654	-1,203	1,869
		P3	1,667(*)	,731	,035	,131	3,203
		self	1,500	,731	,055	-,036	3,036
	P3	Normal	-,167	,731	,822	-1,703	1,369
		P1	-1,333	,731	,085	-2,869	,203
		P2	-1,667(*)	,731	,035	-3,203	-,131
		self	-,167	,731	,822	-1,703	1,369
self	Normal	-3,33E-016	,731	1,000	-1,536	1,536	
	P1	-1,167	,731	,128	-2,703	,369	
	P2	-1,500	,731	,055	-3,036	,036	
	P3	,167	,731	,822	-1,369	1,703	
Dry matter	Normal	P1	,467	,538	,397	-,664	1,598
		P2	-,200	,538	,715	-1,331	,931
		P3	1,833(*)	,538	,003	,702	2,964
		self	1,100	,538	,056	-,031	2,231
	P1	Normal	-,467	,538	,397	-1,598	,664
		P2	-,667	,538	,231	-1,798	,464
		P3	1,367(*)	,538	,021	,236	2,498
		self	,633	,538	,255	-,498	1,764
	P2	Normal	,200	,538	,715	-,931	1,331
		P1	,667	,538	,231	-,464	1,798
		P3	2,033(*)	,538	,001	,902	3,164
		self	1,300(*)	,538	,027	,169	2,431
	P3	Normal	-1,833(*)	,538	,003	-2,964	-,702
		P1	-1,367(*)	,538	,021	-2,498	-,236
		P2	-2,033(*)	,538	,001	-3,164	-,902
		self	-,733	,538	,190	-1,864	,398
self	Normal	-1,100	,538	,056	-2,231	,031	
	P1	-,633	,538	,255	-1,764	,498	
	P2	-1,300(*)	,538	,027	-2,431	-,169	
	P3	,733	,538	,190	-,398	1,864	

Length of pods	Normal	P1	-,467	,663	,490	-1,859	,926
		P2	-1,300	,663	,065	-2,692	,092
		P3	-2,067(*)	,663	,006	-3,459	-,674
	P1	self	-,300	,663	,656	-1,692	1,092
		Normal	,467	,663	,490	-,926	1,859
		P2	-,833	,663	,225	-2,226	,559
	P2	P3	-1,600(*)	,663	,027	-2,992	-,208
		self	,167	,663	,804	-1,226	1,559
		Normal	1,300	,663	,065	-,092	2,692
	P3	P1	,833	,663	,225	-,559	2,226
		P3	-,767	,663	,262	-2,159	,626
		self	1,000	,663	,149	-,392	2,392
	self	Normal	2,067(*)	,663	,006	,674	3,459
		P1	1,600(*)	,663	,027	,208	2,992
		P2	,767	,663	,262	-,626	2,159
	self	self	1,767(*)	,663	,016	,374	3,159
		Normal	,300	,663	,656	-1,092	1,692
		P1	-,167	,663	,804	-1,559	1,226
		P2	-1,000	,663	,149	-2,392	,392
		P3	-1,767(*)	,663	,016	-3,159	-,374
N of pods	Normal	P1	4,983	20,915	,814	-38,958	48,924
		P2	-12,017	20,915	,573	-55,958	31,924
		P3	-71,917(*)	20,915	,003	-115,858	-27,976
		self	-25,750	20,915	,234	-69,691	18,191
	P1	Normal	-4,983	20,915	,814	-48,924	38,958
		P2	-17,000	20,915	,427	-60,941	26,941
		P3	-76,900(*)	20,915	,002	-120,841	-32,959
		self	-30,733	20,915	,159	-74,674	13,208
	P2	Normal	12,017	20,915	,573	-31,924	55,958
		P1	17,000	20,915	,427	-26,941	60,941
		P3	-59,900(*)	20,915	,010	-103,841	-15,959
		self	-13,733	20,915	,520	-57,674	30,208
	P3	Normal	71,917(*)	20,915	,003	27,976	115,858
		P1	76,900(*)	20,915	,002	32,959	120,841
		P2	59,900(*)	20,915	,010	15,959	103,841
		self	46,167(*)	20,915	,041	2,226	90,108
	self	Normal	25,750	20,915	,234	-18,191	69,691
		P1	30,733	20,915	,159	-13,208	74,674
		P2	13,733	20,915	,520	-30,208	57,674
		P3	-46,167(*)	20,915	,041	-90,108	-2,226

Fresh weight	Normal	P1	25,967	279,110	,927	-560,421	612,354
		P2	-104,833	279,110	,712	-691,221	481,554
		P3	-884,050(*)	279,110	,005	-1470,438	-297,662
	P1	self	-333,083	279,110	,248	-919,471	253,304
		Normal	-25,967	279,110	,927	-612,354	560,421
		P2	-130,800	279,110	,645	-717,188	455,588
	P2	P3	-910,017(*)	279,110	,004	-1496,404	-323,629
		self	-359,050	279,110	,215	-945,438	227,338
		Normal	104,833	279,110	,712	-481,554	691,221
	P3	P1	130,800	279,110	,645	-455,588	717,188
		P3	-779,217(*)	279,110	,012	-1365,604	-192,829
		self	-228,250	279,110	,424	-814,638	358,138
	self	Normal	884,050(*)	279,110	,005	297,662	1470,438
		P1	910,017(*)	279,110	,004	323,629	1496,404
		P2	779,217(*)	279,110	,012	192,829	1365,604
	P1	self	550,967	279,110	,064	-35,421	1137,354
		Normal	333,083	279,110	,248	-253,304	919,471
		P1	359,050	279,110	,215	-227,338	945,438
P2		228,250	279,110	,424	-358,138	814,638	
P3		-550,967	279,110	,064	-1137,354	35,421	
Minor defects	Normal	P1	2,300	4,656	,627	-7,483	12,083
		P2	-3,600	4,656	,449	-13,383	6,183
		P3	-15,083(*)	4,656	,005	-24,866	-5,300
	P1	self	-3,067	4,656	,518	-12,850	6,716
		Normal	-2,300	4,656	,627	-12,083	7,483
		P2	-5,900	4,656	,221	-15,683	3,883
	P2	P3	-17,383(*)	4,656	,002	-27,166	-7,600
		self	-5,367	4,656	,264	-15,150	4,416
		Normal	3,600	4,656	,449	-6,183	13,383
	P3	P1	5,900	4,656	,221	-3,883	15,683
		P3	-11,483(*)	4,656	,024	-21,266	-1,700
		self	,533	4,656	,910	-9,250	10,316
	self	Normal	15,083(*)	4,656	,005	5,300	24,866
		P1	17,383(*)	4,656	,002	7,600	27,166
		P2	11,483(*)	4,656	,024	1,700	21,266
	P2	self	12,017(*)	4,656	,019	2,234	21,800
		Normal	3,067	4,656	,518	-6,716	12,850
		P1	5,367	4,656	,264	-4,416	15,150
P2		-,533	4,656	,910	-10,316	9,250	
P3		-12,017(*)	4,656	,019	-21,800	-2,234	
Serious defects	Normal	P1	,350	3,722	,926	-7,470	8,170
		P2	-3,667	3,722	,338	-11,487	4,153
		P3	-4,183	3,722	,276	-12,003	3,637
	P1	self	-2,017	3,722	,595	-9,837	5,803
		Normal	-,350	3,722	,926	-8,170	7,470
		P2	-4,017	3,722	,295	-11,837	3,803
	P2	P3	-4,533	3,722	,239	-12,353	3,287
		self	-2,367	3,722	,533	-10,187	5,453
		Normal	3,667	3,722	,338	-4,153	11,487
	P3	P1	4,017	3,722	,295	-3,803	11,837
		P3	-,517	3,722	,891	-8,337	7,303

	P3	self	1,650	3,722	,663	-6,170	9,470
		Normal	4,183	3,722	,276	-3,637	12,003
		P1	4,533	3,722	,239	-3,287	12,353
		P2	,517	3,722	,891	-7,303	8,337
	self	self	2,167	3,722	,568	-5,653	9,987
		Normal	2,017	3,722	,595	-5,803	9,837
		P1	2,367	3,722	,533	-5,453	10,187
		P2	-1,650	3,722	,663	-9,470	6,170
		P3	-2,167	3,722	,568	-9,987	5,653
Diseases	Normal	P1	1,550	1,028	,149	-,609	3,709
		P2	,233	1,028	,823	-1,926	2,392
		P3	,250	1,028	,811	-1,909	2,409
		self	1,033	1,028	,328	-1,126	3,192
	P1	Normal	-1,550	1,028	,149	-3,709	,609
		P2	-1,317	1,028	,216	-3,476	,842
		P3	-1,300	1,028	,222	-3,459	,859
		self	-,517	1,028	,621	-2,676	1,642
	P2	Normal	-,233	1,028	,823	-2,392	1,926
		P1	1,317	1,028	,216	-,842	3,476
		P3	,017	1,028	,987	-2,142	2,176
		self	,800	1,028	,446	-1,359	2,959
	P3	Normal	-,250	1,028	,811	-2,409	1,909
		P1	1,300	1,028	,222	-,859	3,459
		P2	-,017	1,028	,987	-2,176	2,142
		self	,783	1,028	,456	-1,376	2,942
	self	Normal	-1,033	1,028	,328	-3,192	1,126
		P1	,517	1,028	,621	-1,642	2,676
		P2	-,800	1,028	,446	-2,959	1,359
		P3	-,783	1,028	,456	-2,942	1,376
Date of first flowerl	Normal	P1	-5,500(*)	1,893	,009	-9,476	-1,524
		P2	-1,917	1,893	,325	-5,893	2,060
		P3	,167	1,893	,931	-3,810	4,143
		self	-6,833(*)	1,893	,002	-10,810	-2,857
	P1	Normal	5,500(*)	1,893	,009	1,524	9,476
		P2	3,583	1,893	,075	-,393	7,560
		P3	5,667(*)	1,893	,008	1,690	9,643
		self	-1,333	1,893	,490	-5,310	2,643
	P2	Normal	1,917	1,893	,325	-2,060	5,893
		P1	-3,583	1,893	,075	-7,560	,393
		P3	2,083	1,893	,286	-1,893	6,060
		self	-4,917(*)	1,893	,018	-8,893	-,940
	P3	Normal	-,167	1,893	,931	-4,143	3,810
		P1	-5,667(*)	1,893	,008	-9,643	-1,690
		P2	-2,083	1,893	,286	-6,060	1,893
		self	-7,000(*)	1,893	,002	-10,976	-3,024
	self	Normal	6,833(*)	1,893	,002	2,857	10,810
		P1	1,333	1,893	,490	-2,643	5,310
		P2	4,917(*)	1,893	,018	,940	8,893
		P3	7,000(*)	1,893	,002	3,024	10,976

Date of first pod	Normal	P1	-7,167(*)	1,967	,002	-11,299	-3,034
		P2	-2,333	1,967	,251	-6,466	1,799
		P3	-2,167	1,967	,285	-6,299	1,966
		self	-7,583(*)	1,967	,001	-11,716	-3,451
	P1	Normal	7,167(*)	1,967	,002	3,034	11,299
		P2	4,833(*)	1,967	,024	,701	8,966
		P3	5,000(*)	1,967	,020	,868	9,132
		self	-,417	1,967	,835	-4,549	3,716
	P2	Normal	2,333	1,967	,251	-1,799	6,466
		P1	-4,833(*)	1,967	,024	-8,966	-,701
		P3	,167	1,967	,933	-3,966	4,299
		self	-5,250(*)	1,967	,016	-9,382	-1,118
	P3	Normal	2,167	1,967	,285	-1,966	6,299
		P1	-5,000(*)	1,967	,020	-9,132	-,868
		P2	-,167	1,967	,933	-4,299	3,966
self		-5,417(*)	1,967	,013	-9,549	-1,284	
self	Normal	7,583(*)	1,967	,001	3,451	11,716	
	P1	,417	1,967	,835	-3,716	4,549	
	P2	5,250(*)	1,967	,016	1,118	9,382	
	P3	5,417(*)	1,967	,013	1,284	9,549	

Based on estimated marginal means

* The mean difference is significant at the ,05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Pairwise Comparisons

Dependent Variable	(I) Cv	(J) Cv	Mean Difference (I-J)	Std. Error	Sig.(a)	95% Confidence Interval for Difference(a)	
						Upper Bound	Lower Bound
Dead Stems	O	R	,133	,462	,776	-,838	1,105
	R	O	-,133	,462	,776	-1,105	,838
Dry matter	O	R	,333	,340	,341	-,382	1,049
	R	O	-,333	,340	,341	-1,049	,382
Pod length	O	R	3,220(*)	,419	,000	2,339	4,101
	R	O	-3,220(*)	,419	,000	-4,101	-2,339
N of Pods	O	R	14,760	13,228	,279	-13,031	42,551
	R	O	-14,760	13,228	,279	-42,551	13,031
Fresh Weight	O	R	510,267(*)	176,524	,010	139,403	881,131
	R	O	-510,267(*)	176,524	,010	-881,131	-139,403
Minor defects	O	R	-2,400	2,945	,426	-8,587	3,787
	R	O	2,400	2,945	,426	-3,787	8,587
Serious defects	O	R	-1,740	2,354	,469	-6,686	3,206
	R	O	1,740	2,354	,469	-3,206	6,686
Diseases	O	R	,653	,650	,328	-,712	2,019
	R	O	-,653	,650	,328	-2,019	,712
Date of first flor	O	R	2,967(*)	1,197	,023	-,452	5,481
	R	O	-2,967(*)	1,197	,023	5,481	-,452
Date of first pod	O	R	-1,567	1,244	,224	-4,180	1,047
	R	O	1,567	1,244	,224	-1,047	4,180

Based on estimated marginal means

* The mean difference is significant at the ,05 level.

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

The date of first pod

Duncan

PortaEnx	N	Subset	
		2	1
Normal	6	40,5000	
P3	6	42,6667	
P2	6	42,8333	
P1	6		47,6667
self	6		48,0833
Sig.		0,276	0,835

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = 11,606.

a Uses Harmonic Mean Sample Size = 6,000.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c Alpha = 0,05.