Chapter 17

Grafting horticultural plants as technique for the control of soil pathogens

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1. Concepts about grafting onto herbaceous species

1.1 Introduction and historic evolution of herbaceous grafting

Herbaceous grafting is a current crop technique in horticulture. It comes from the East. As Janick (1986) reported, grafting onto herbaceous species was already described in the A.D. fifth century in China. In the seventeenth century in Korea, Hong (1643-1715) wrote a treatise where he reported a grafting technique whereby he joined four rootstocks to only one gourd stem, in two stages, removing some of the fruits, leaving only one or two per plant, in order to obtain big size gourds which would serve for storing rice grains (Janick, 1986; Lee and Oda, 2003).

As early as the twentieth century, other historical references about the use of grafting in cucurbits highlight their interest. During the twenties, the bottle gourd (*Lagenaria siceraria* Standl.) was used as watermelon rootstocks (*Citrullus lanatus* Matsum et Nakai) to fight against the decrease in yield due to the incidence of soil pathogens associated with the repetition of crops in rotation (Lee, 1994). In 1947 grafting cucumber and melon plants onto *Cucurbita ficifolia* was first carried out in Holland. In France, the first reference about melon grafting reported the use of *Benincasa cerifera* as rootstock and dates from 1959.

The development and use of plastics in agriculture led to an increase of yield in plants in specialised nurseries and permitted the production and distribution of grafted plants in countries such as Korea or Japan. In the sixties, grafting was introduced in the commercial production of cucumber and tomato crops in these countries. At the end of the eighties and beginning of the nineties of the last century, this technique was extended to horticultural species like watermelon, cucumber, melon, tomato and aubergine (Oda, 1993).

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The development of this technology in Spain began in Valencia at the end of the seventies and it was developed in Valencia and Almería through the eighties. Currently, in Almería, more than 95 % of the cultivated watermelon area is grafted (Camacho and Fernández-Rodríguez, 2000), and the number of hectares that use this crop technique is increasing more and more in order to guarantee the production of species like tomato, melon or cucumber.

In the following table, a summary of the data obtained about the percentage of grafted plants produced under protected horticultural conditions in different countries is shown, according to the data obtained by Traka-Mavrona *et al.*, 2000. Oda, 1993. Lee and Oda, 2003. Miguel *et al.,* 2007. Diánez *et al.,* 2008. Huitrón and Camacho, 2008. As well as the non published data by the United Nations Industrial Development Organization, to prepare "Alternative to Methyl Bromide" projects which this United Nations agency is developing in different developing countries, under the Montreal Protocol.

Table 1. Percentage of grafted plants in protected horticulture by countries

Country	Watermelon	Melon	Cucumber	Tomato	Pepper	Aubergine
Japan	93-98 %	30-42 %	72-96 %	32-48 %	*	50-94 %
Korea	98 %	83-95 %	95 %	5 %	5 %	2 %
Greece	100 %	40-50 %	5-10 %	2-3 %	*	*
Spain	98 %	3 %	I	10 %	I	I
Italy	30 %	*	*	*	*	*
Cyprus	80 %	*	*	*	*	*
France	*	I	3 %	30 %	*	*
Holland	*	*	I	50 %	*	*
Israel	40-70 %	*	*	*	*	*
Egypt	I	I	*	*	I	*
Jordan	25 %	*	*	*	*	*
Tunisia	*	I	*	*	*	*
Morocco	25-50 %	*	*	*	*	*
Mexico	I	I	*	I	*	*
Guatemala	I	*	*	*	*	*
Honduras	20 %	I	*	*	*	*

I--- It begins the technique at commercial level. Without comparative data
*-- Non available data.

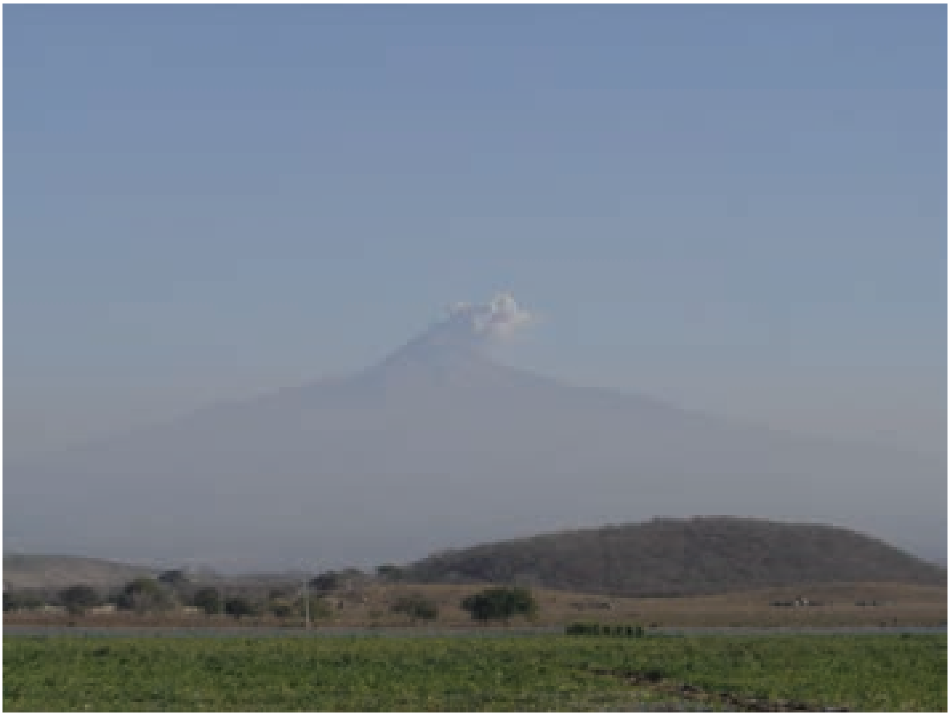

Photo 1. Grafted watermelon in Colima – Mexico

In Japan, 500 million plants are grafted yearly. A 10 % (40 % in watermelon) of these plants are grafted mechanically (Hassell *et al.,* 2008). In the USA, 40 million tomato plants are grafted for hydroponic crops and scarcely 400000 watermelon plants. Currently, in Mexico, there are 1000 watermelon hectares with grafted plants outdoors, to avoid Fol race 3, in addition to 1000 watermelon hectares and 100 melon hectares (Davis *et al.,* 2008).

1.2 The purpose of grafting

In horticulture, the main purpose of grafting is to create the possibility of cultivating plants that are sensitive to some diseases in a contaminated soil. The system consists of using a rootstock which belongs to other varieties, species, and even other genus of the same crop family. The rootstock must be resistant to the disease that we want to avoid (Louvet, 1974).

The rootstock remains healthy which can provide the nutritive solution to the plant, at the same time it is developed, it also provides the necessary photoassimilates which are prepared in the aerial part (cultivar). When using this technology to prevent soil pathogens, the rootstock root system is left alone and also the aerial part corresponding to the variety. The rootstock-variety interaction modifies, in most cases, the behaviour of the same as a consequence of different reactions such as incompatibility, change in the tolerance to some factors of climate and soil, growth habits, flowering, fruit size, content of soluble solids of the same, flesh firmness, etc.

As it has been previously mentioned, the main use of grafting in horticulture is the control of diseases and soil "fatigue" (Yu-Jin Quan 2000, 2001); but this technology can be used with other purposes, such as to prolong the crop cycle and other procedures in disciplines such as: vegetal propagation, vegetal enhancement, phytopathology, vegetal physiology and special phytotechnics.

In the crop of herbaceous species through grafting, the main useful characteristics of the rootstock are intended to be used, whose results are related with:

- Increase of vigour and possibility of cropping a longer cycle (Louvet, 1974; Choi *et al*., 1980; Ogbuji, 1981; Buitelaar, 1987; Vergniaud, 1990; Miguel *et al*., 2007).
- Increase of yield (Miguel, 1993; Miguel *et al.,* 2007; Alexandre *et al.,* 1997b; Gamayo and Aguilar, 1998; Sánchez, 2000; Trionfetti *et al.,* 2002; Davis *et al.,* 2008).
- Resistance and/or tolerance to diseases and soil pests (Louvet and Peyriere, 1962; Buitelaar, 1987; Messiaen *et al.,* 1991; Gómez, 1993; Miguel *et al.,* 2007; Cohen *et al.,* 2000; Trionfetti *et al.,* 2002; Lee and Oda, 2003; González, *et. al.;* 2008; King *et al.,* 2008; Davis *et al.,* 2008; Kubota *et al.,* 2008).
- Tolerance to abiotic stresses. For example, grafted melon on RS-841 (*C. maxima x C. moschata*) better tolerates low soil temperatures, allowing earlier plantations (Buitelaar, 1987; Vergniaud, 1990; Yu-XianChang *et al.,* 1997; Lee, 2003). Some rootstocks allow the enhancement of tolerance to tomato salinity (Estan *et al.,* 2005) or melon (Colla *et al.,* 2006) or to flooding (Liao-ChungTa; Lin-ChinHo 1996).

- Increase in the calibre of the fruit (Miguel, 1993; Miguel *et al.,* 2007; Camacho, 1999; Sánchez, 2000; Traka-Mavrona *et al.,* 2000; Trionfetti *et al.,* 2002).
- Modification of the attributes of fruit quality (Choi *et al.,* 1980; Lee, 1989; Davis *et al*., 2008).
- Combination of materials of ornamental value as it occurs with cactus of *Gymnocalicium mihanovichii* species var. friedichii Werd. Hort, whose world demand is estimated to be at around 10 million plants annually (Lee and Oda, 2003).

1.3. Graft physiology

The changes produced when grafting a vegetal species, in relation with aspects such as growth and development are the following:

a) Graft union

In herbaceous species, Andrews and Márquez (1993) studied the structural changes produced when grafting tasks were made: in the grafted area, the broken cells collapse and a necrotic layer is developed which will later disappear. Then, the living cells of the rootstock and the variety are developed on the necrosed area. Through the cell division a callus made of parenchymatous cells is formed, which causes the break and invasion of the entire necrotic layer. In the process, the resistance to the traction of the grafting point increases due to the physical union between both vegetal materials (rootstock-variety). From the parenchymatous cells, a new cambium is generated, and a xylem as well as a secondary phloem is differentiated, which allow the vascular connection between the grafted materials.

The union of rootstock and variety is formed by cells which have been developed after grafting has been made. These cells are produced by the two vegetal materials which we graft, and they keep their identity so that they do not mix their cellular contents.

Two stages can be distinguished in the union process. The first is a compatibility reaction, the most characteristic process of which is an active cell division in the adjacent tissues, causing an increase of tracheids. The second stage completes the union, because the vascular continuity by differentiation of the tracheids is restored, such tracheids having been formed in the previous stage in vascular elements (Lindsay *et al.,* 1974). In time, the differentiation of both stages can be 3-4 days each.

In some horticultural species, especially in cucurbits, the stem shows an internal cavity in the hypocotyl and epicotyl and, in most of the cases, it also shows six vascular bundles in a well-defined position. The complete union of these six vascular bundles is sometimes impossible, depending on the grafting technique we use, but the final purpose is the achievement of a quick and complete vascular union (Oda *et al.,* 1993).

b) Graft compatibility

Compatibility is defined as the capability that two different plants have to join and develop satisfactorily throughout the whole cycle as a composite plant (Miguel, 1993).

There is not a clear distinction between a compatible or incompatible graft, because it can include species with a close morphological and physiological relation and, therefore, they join easily and also species that are totally incompatible; but between both of them, an intermediate adjustment could be fixed, there are some plants that join, but as time goes by, show disorders, whether in the union or in the growth habit (Hartmann and Kester, 1991). In general, compatibility is related with taxonomic affinity, but it has significant exceptions. Usually, the incompatibility symptoms do not appear until the plant is well developed. The most obvious are (Hartmann and Kester, 1991):

- High percentage of failures in the graft.
- Excessive development of the union (*miriñaque*) over or under it.
- Yellowing, curling and lack of growth of foliage.
- Premature death of plants.
- Marked differences in the growth rate between rootstock and variety.
- Breaking of the grafting union.

Even if some of these symptoms appear, it does not necessary mean that the union is incompatible, but it can be a consequence of unfavourable environmental conditions, presence of diseases or a bad grafting technique.

The physiological incompatibility can be due to, amongst other things, the lack of cell recognition, response to the injury, the role of phytoregulators, if any, or incompatible toxins (Lee and Oda, 2003).

In cucurbits, it seems that there is a cell recognition mechanism where substances such as phytohormones, are involved. Such substances are released by the injured tissues and affect the cambium activity in the grafting area. Thus, in the Cucumis-Cucumis union the development of phloem in the grafting area is higher than the combination Cucumis-Cucurbita (Tiedermann, 1989).

In any case, it is especially important to highlight how incompatibility can change depending on the grafting techniques and the crop environments, as has been reported by Lee (1994).

Furthermore, the type of cultivar within a specific species, as it occurs in melon, can mean that a rootstock is compatible with some types and incompatible with others.

2. The control of soil pathogens with the use of grafting

Grafting is an eco-compatible technique, which does not generate residues and create jobs wherever it is carried out. This technique is justified from the phytosanitary point of view and according to the bibliography and trials made, in solaneaceous plants, (tomato, pepper and aubergine) and in cucurbits (watermelon, melon and cucumber), the grafting technique controls the incidence and development of the diseases listed: (Now, when we refer to solanaceaus plants, as later when we will refer to cucurbits, the section shall begin with a series of bibliographic references that justify the use of the grafting technique as a defense of this species against soil diseases).

2.1. Solanaceous plants

(Lacasa *et al.,* 2008; García Jiménez *et al.,* 2007; Tello *et al.,* 1998; Besri M, 2004; Black *et al.,* 2003; Miguel A, 2002; Romano and Paratore, 2001; Camacho and Fernández, 2000; Bradley J. 1968).

2.1.1. Tomato

a) Fusarium wilt: caused by *Fusarium oxysporum* f. sp. *lycopersici*

This fungus, which can survive in the soil, causes a vascular disease. It penetrates into the plant through the roots and is spread quickly through the xylem, producing a brown colouring in the conductive vessels, from the roots to the leaf petioles. The initial symptoms are the nerves thinning and the flagging aspect of petioles. The lower leaves turn yellow (sometimes only half of the leaf) and later they wilt and dry, although remain attached to the plant. This disease does not affect the whole plant equally, and diseased branches can appear while others remain healthy. The main roots and the stem base show vascular necrosis (García-Jiménez, 2007). Plant growth stops, fruits ripe prematurely and the plant can even die. There are three identified races of this pathogen and there are several commercial varieties with resistance to one or two of them, which are the more common.

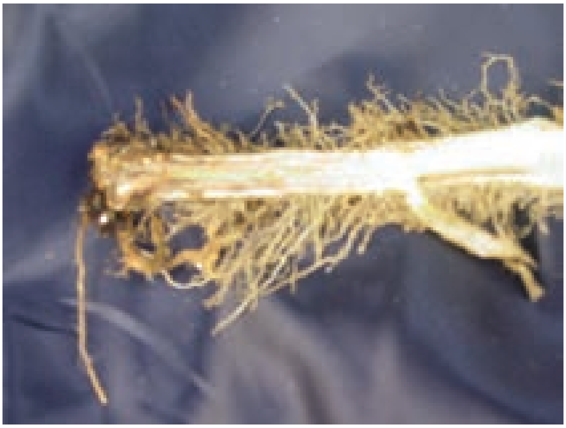
Photo 2. Fusarium in tomato ***cv*** Pitenza

b) Fusarium wilt: caused by *Fusarium oxysporum* f.sp. *radicis lycopersici*

It is significant mainly in greenhouses and crops without soil. It causes the rot of the cortical parenchyma of roots and progress through the conductive vessels of the same, up to the base of the stem. In the neck of the affected plants a necrotic chancre appears which is spread pointed toward the top (Tello and Lacasa, 1988). A general wilt and yellowing of leaves is produced, which begins in the base and spreads towards the tip (García-Jiménez 2007). There are some commercial varieties with resistance to this pathogen (Messiaen *et al.,* 1991).

Photo 3. Grafted tomato affected by Fusarium due to franqueamiento (emission of adventitious roots by the harvested cultivar) of the variety.

c) Corky root: caused by the fungus *Pyrenochaeta lycopersici*

It attacks the root system and, sometimes, the stem base. It produces an early loss of the smaller roots and the main roots become severely corky, with cracks along their length. As a consequence of this, plants show little development, wilt and a drying of the basal leaves. Yield is strongly reduced. There are some varieties with a certain tolerance, but a real protection is only given by grafting onto some rootstocks (García-Jiménez 2007).

d) Pepino mosaic virus, (PepMV)

It seems to be responsible for the alteration known as "tomato collapse". In the fruits of the plants affected by this disease, some mottles are appreciated and mosaics of different green tonalities can be observed in the leaves. The wilting and death of the plants, which is known as "Collapse" or "Sudden death", has only been detected when the virus is associated in the aerial part of the plant and *Olpidium brassicae* in the roots under specific environmental conditions, and it has less incidence and is even not found in greenhouses under a controlled temperature. The solution to this problem has been found when grafting is used on very vigorous rootstocks. As occurs with other diseases, *franqueamiento* (emission of adventitious roots by the harvested cultivar) of the grafted plant means that graft does not take effect. Resistant varieties to "collapse" are not known (Jordá M.C, 2007; Lacasa et al 2008).

Photo 4. Beginning of collapse in tomato

2.1.2. Pepper

a) Tristeza: disease caused by the *Phytophtora capsici* fungus

The attack of this fungus on the pepper plant can appear in any vegetal state, although the most critical phenological state is at the beginning of fructification. At the beginning it is almost imperceptible because the plant does not show symptoms, but later dark spots appear on the plant's neck, and when these spots affect the entire neck a vascular disorder is produced which blocks the sap circulation. In the latter stages, the plant shows wilting without defoliation and early ripening of fruits (García-Jiménez, 2007).

2.1.3. Tomato, pepper and aubergine

a) Verticillium wilt: disease caused by *Verticillium dahliae*

The primary symptoms that can be seen are interveinal chlorosis in the basal leaves, and then yellowing in the tip of leaflets which will dry up. Likewise, this occurs with Fusarium wilt, described for tomatoes, and at midday hours a plant dehydration of the aerial part is observed, recovering at night, until the permanent wilt is produced. These symptoms usually only appear in a part of the plant, but later spread to the whole plant. The vascular system presents a red-brown colour which advances from the base to the tip of the plant. The attack is produced with mild temperatures of 20-25 ºC. This fungus can survive in the soil for several years and also has a wide host range, including cultivated plants as well as weeds. Constant mono-cropping causes aggressive attacks to the plants (García-Jiménez, 2007). In the tomato, the Ve gen provides a good resistance but in the case of the aubergine, no resistant variety is known.

b) Bacterial wilt: caused by *Ralstonia solanacearum*

It causes a systemic disease, which is the main bacterial problem of these crops in mild and tropical areas, but it is not very widespread in Europe, where it is considered as a quarantine pathogen. In the last ten years, foci of this bacteriosis have been described in most of the European countries, including Spain (López, M.M., 2007). At first, unilateral wilting of leaves is identified and roots appear in the stem. The plant dies very soon. The vascular system turns brown. If transversal cuts are made in the stem, a milky exudate can be seen which does not appear if the vascular disease is caused by fungi.

R. solanacearum can survive for many years in the soil and also in weeds, aquatic plants and watercourses (López and Biosca, 2005). Some tomato varieties are resistant. *Ralstonia* has few control alternatives. Methyl bromide is not effective and neither is the combination of 1,3 Dicholopropene + Chloropicrine, however, grafting is effective (Grimault and Prior, 1994, quoted by King *et al.,* 2008).

2.2. Cucurbits

(García Jiménez *et al.,* 2007; Fernández-Rodríguez *et al.,* 2002; Hirai *et al.,* 2002; Trionfetti *et al.,* 2002; Cohen *et al.,* 2000; Miguel *et al.,* 2007; Eldelstein *et al.,*1999; Baixauli *et al.,* 1999; Alexandre *et al.,* 1997a; 1997b; Morra, 1997; Pivonia *et al.,* 1996; Gómez, 1993; Messiaen *et al.,* 1991; García Jiménez *et al.,*1990, 1991; Vergniaud, 1990; Yoshida *et al.,* 1987; Buitelaar, 1987; Alabouvette *et al.,* 1974; Louvet, 1974; Chavagnat *et al.,* 1972; Suzuki, 1972; Louvet y Peyriere, 1962; Gronewegen, 1953).

2.2.1. Watermelon

a) Fusarium wilt: caused by *Fusarium oxysporum* f. sp. *niveum*. (FON)

This fungus causes the massive death of plants in most of the producing areas of the world. Three races of the pathogen are known. The FON penetrates the roots and is localised in the woody vessels of the plant. Due to the blocking and necrosis, it is difficult to transport water and nutrients, thus leaves and sprouts wilt. At the beginning, only yellowing and side wilt are produced in the plant, sometimes in only one sprout. Lesions in the conductive vessels appear as spots that spread from the shoot to the tip. Then, the disease is spread to the other shoots, infesting the whole plant which finally dies. In the roots, brown spots are appreciated throughout the vascular bundles and reddish gummy secretions appear next to the plant's neck. In soils highly infested by the pathogen, plants can wilt and die before reaching the adult stage. The fungus can survive in the soil for more than ten years without cultivating watermelon, as resistant organs (chlamydospores) or colonizing roots of adventitious plants or of other crops to which it does not cause damage (García-Jiménez, 2007).

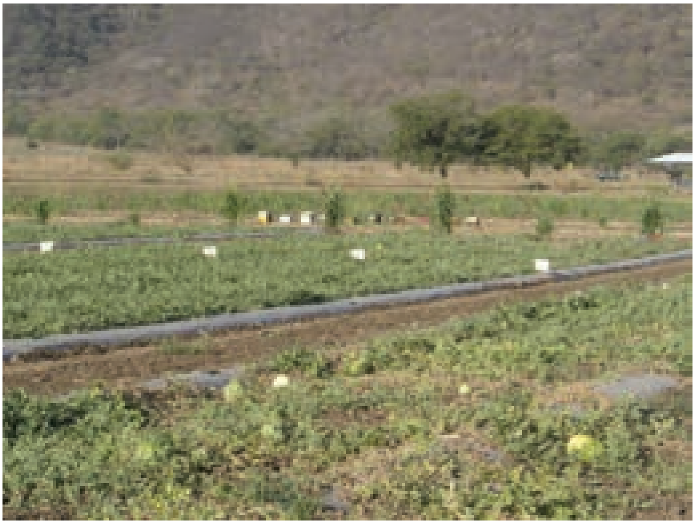

Photo 5. Trials of grafted watermelon against non grafted watermelon in plots infested by MNSV and Olpidium bornovanus. Close-up Photos of non-grafted watermelons

2.2.2. Melon

a) Fusarium wilt in melon: causal pathogen is *Fusarium oxysporum* f. sp. *melonis*.

This is considered as being the main soil pathogen of melon in many countries. Likewise with the case of watermelon, this Fusarium is vascular. To date, four races of this pathogen are known, 0, 1, 2 and 1-2. In 2000 Gómez and Tello, reported for the first time the presence of the physiological race 1-2 in Spain, and the detection of isolates belonging to races 0 and 1 of the pathogen associated to symptoms of "Wilt type", which meant a world first. The syndrome of the disease includes two types of symptoms: "Type Yellows": which is characterised by venation yellowing followed by a generalised limb yellowing, together with necrotic lesions in stems and petioles, gummy exudations and subsequent plant death. "Type Wilt": which is characterised by a plant wilt, not yellowing, which is developed from the tip of the stems to the base of the plant (Mas and Risser, 1966). There are some commercial varieties of melon with resistance to one or several of them (González-Torres *et al.,* 1994).

Photo 6. Trials of grafted melon against non grafted in plots infested by Fom. The non grafted plots can be observed by the great number of dead plants and the differences in the development

Photo 7. Detail of Fom in the conductive bundles of melon

2.2.3. Cucumber

a) Fusarium wilt of cucumber: Caused by *Fusarium oxysporum* f. sp. *cucumerinum*

This disease appears in all the development states of the cucumber, even in seedlings. First, symptoms appear in the basal leaves, which begin to wilt. Also it is very common to find leaf yellowing, wilt and progressive drying up of limbs. The vessels show a brown colour. The adjacent tissues to the same undergo necrosis and gummy exudates are appreciated outside with different tonalities which vary from pinkish to reddish, and when disease affects the whole vascular system, it causes its death. (García-Jiménez, 2007).

b) Root and stem base rot: caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* fungus

The first symptoms in the Mediterranean basin are observed in autumn. A rot of the neck, usually on only one side of the stem, is developed, which varies from pale green to amber or brown. As development of the rot increases, a white fungal growth is appreciated in the affected tissues. The plants that show these symptoms reduce their growth, wilt and die a few weeks after the symptoms have appeared (García-Jiménez, 2007).

c) Cucumber Leaf Spot Virus: disease caused by Cucumber Leaf Spot Virus (CLSV)

Its behaviour is similar to that presented by the necrotic spot virus, which we shall deal with later. Chlorotic spots, whose central part are brown, are observed on the leaves of the plants affected by this disease, followed by a process of necrosis. In general, it causes plant dwarfism and flowering delay. It is transmitted by seed and through the *Olpidium bornovanus* fungus (Jordá, M.C 2007).

2.2.4. Watermelon and melon

a) Collapse or sudden death of melon and watermelon crops

There are several pathogens involved in processes that end with wilting and plant death in melon and watermelon crops. Under the ambiguous terms of “collapse” or “sudden death” a series of conditions are included, to which the final destination leads to the sudden death of the plant, generally in advanced development states, this death being caused by a strong imbalance between the hydric needs of the aerial part and the actual amount of water it receives.

In the list of soil diseases made by Gómez in Almería between the years 1987 and 1992, it was decided that the main cause responsible for the death of melon plantations were two pathogens of telluric origin: the Fusarium wilt (causal agent *Fusarium oxysporum* f. sp. *melonis*) being the most frequent, with a presence of 45,1 % in the sampled greenhouses, followed by the melon necrotic spot virus MNSV with a presence of 34,3 % (Gómez, 1993).

Melon necrotic spot virus (MNSV). The association of sudden death to the melon necrotic spot virus (MNSV), has been described in several works (Gómez and Velasco, 1991; Gómez *et al.,* 1993a, b). Its main vector is the *Olpidium bornovanus* fungus, which has been found in a high percentage of greenhouses in Almería. Although this disease was first associated with protected melon crop, it now also affects crops of outdoor melon, watermelon, cucumber and other cucurbits (Jordá,M.C, 2007).

Firstly, small chlorotic spots of approximately 1-2 mm diameter appear in the leaves which become necrotic spots and can even pierce them. The appearance of necrosis is very typical in the leaf venation, forming a grille. It causes stretch marks on the plant stem and neck and, finally, a sudden wilt and subsequent death. A rough skin, spotted with woody marks and internal mottle is appreciated in fruits. It is also transmitted by seed.

b) Monosporascus cannonballus, Acremonium cucurbitacearum

With the same effect, of “collapse or sudden death” in the Valencian Community, as well as in other areas of melon crops (Texas, Arizona, California, Korea, Israel) this is attributed to fungi of Monosporascus genus (Lobo, 1990; García-Jiménez *et al.,* 2007; Beltrán *et al.,* 2008)), possibly associated with others of the Acremonium genus. These fungi are the most significant and have the highest incidence in Spain, and it is very common that they appear together in affected plots. *M. cannonballus* has been described in many countries, mainly in arid and hot areas. The typical symptoms of Monosporascus are round black bulges, which are visible to the naked eye, which correspond to fungus perithecia. In the observations made in Spain, these black bulges appear only in almost dead plants. Before this, a decline of the plant is produced, a gradual advance that affects the youngest leaves and branches, thus the affected plants decline and die prematurely (García Jiménez 2007). All the cucurbits are susceptible to Monosporacus, but while the fungus is easily isolated from the melon and watermelon roots, it is difficult to find it in Cucurbits (Beltrán *et al.,* 2008). Other wild plants of the same or different families can serve as its hosts, although only melon and watermelon are affected by the fungus. Ascospores are considered as the primary inoculum. Solarization is not effective for its control, because this is a thermophile organism (Martyn 2007).

Photo 8. Root of gourd infected by *Rhizoctonia* sp.

A. cucurbitacearum was originally described in Spain and later it has been detected in the United States (California, Oklahoma and Texas) and Italy. This is a specific pathogen of cucurbits to which it can affect in different degrees, depending on the species (Armengol *et al.,* 1998). It acts causing necrosis of small roots and roots browning in an uninterrupted process which begins in the first development stages of the plant (Alfaro-García *et al.,* 1996).

Photo 9. Root of gourd infects primeramente for *Rhizoctonia* sp., then *Monosporascus cannoballus* as saprofito

2.3. Solanaceae and Cucurbits

2.3.1. Tomato, pepper, aubergine, watermelon, melon and cucumber

a) Nematodes: Mainly in Spain, of the ***Meloidogyne*** genus

They are translucent and microscopic worms, with different shapes and sizes, some of them are elongated and cylindrical and others are pear or lemon shaped. In general, they are in the soil and affect the root of several vegetal species. Taking into account the different genera of nematodes described, Meloidogyne is practically the only one that affects cucurbits (Verdejo and Sorribas, 1994).

In the field, plants affected by nematodes usually appear grouped, forming small areas where the plants can die in the first development stages or present a stunted development with a tendency to wilt easily due to hydric imbalances. When these plants are pulled up, some bulges with an irregular shape and size can be appreciated on the roots, known as galls or knots, which appear due to hypertrophy and hyperplasia of the tissues in the feeding area of nematodes. In advanced states of attack, browning and rot of the affected areas is produced. The usual means of transmission of this disease are irrigation water and farming implements. Many tomato varieties and hybrids have a gene, the Mi; which provides resistance to *Meloidogyne incognita*, *M.arenaria* and *M. javanica*, but this resistance is not effective when the soil temperature exceeds 29 ºC. (Kubota *et al.,* 2008). Also, races of Meloidogyne have been identified which are able to exceed the resistance of the Mi gen. The Mi 3 gen is possibly resistant to temperatures higher than 30 ºC (King *et al.,* 2008). Nematodes are not a problem in non grafted watermelon crops, because this species shows a certain resistance. However, in watermelons grafted onto pumpkin, they sometimes cause significant damages.

The nematode attacks in peppers are not so obvious as in tomato or pumpkin crops, however this is one of the most important phytopathological problems when crops are repeated frequently. There are pepper varieties and rootstocks with resistance to nematodes, but this resistance is easily overcome if they are cultivated repeatedly, without soil disinfection.

b) Soil fatigue

The repetition of the same crop gives rise to a decrease in yields and a lack of plant vigour, even when there is no evident pathological cause. This phenomenon is known as “soil fatigue”, and it is more appreciated in some species, probably due to the root exudate of toxic substances for the same species. In addition to crop rotation and the soil disinfection, another method to avoid this effect is grafting onto less sensitive species (Yu-JingQuan 2001).

There are big differences in the autotoxic potential of the different cucurbits, watermelon, melon and cucumber being more sensitive and *Cucurbita moschata*, *Lagenaria leucantha* and *Luffa cilíndrica* being less so. (Yu-JingQuan, 2000).

Amongst the Solanaceae, the pepper is probably the crop most sensitive to “soil fatigue”, but in this case grafting cannot be as effective as in cucurbits, because necessarily it has to be grafted onto its own species.

3. Resistance to soil borne diseases

The resistance to Fusarium is located in the set root-hypocotyl. When there is a vascular pathogen in the soil, this can contaminate the grafted plant if the plant has emitted adventitious roots. In other cases, the resistance is due to the synthesis of several substances that produce tolerance to Fusarium and once they have been synthesized in the rootstock roots, are translocated to the variety, via xylem (Biles *et al.,* 1989); this fact could justify that plants with two root systems (whether by *franqueamiento* or by their own grafting) sometimes offer a resistance comparable with that of the grafted plant that has only the root system of the rootstock. Over the graft, the conductive vessels coming from the variety’s root undergo necrosis, but the continuity of those of the rootstock is enough to guarantee appropriate water and nutrient supply for the plant’s needs. When the two root systems are left or the *franqueamiento* of the variety has been produced, even though grafting provides resistance to a pathogen another pathogen can penetrate to which rootstock does not guarantee resistance over grafting. This is the case of the grafted watermelon to which the variety root has not been cut (Miguel et al 2007); the resistance to Fusarium wilt remains, but immunity against pathogens like MNSV is not guaranteed. The activity of the substances related with resistance to diseases can vary during the different development stages of the grafted plants (Padgett and Morrison, 1990).

The tolerance to Monosporascus of the hybrids *C. maxima x C. moschata* is due to their roots not stimulating the germination of fungus spores (Beltrán *et al.,* 2008). It seems that resistance is due to a lack of recognition between host and pathogen. The population of ascospores in soil cultivated with grafted watermelon, is kept or decreases.

The resistance to *Ralstonia* seems to be due to the difficulty of spreading the bacteria in the lower part of the stem of the resistant plant (Grimault,-and Prior, 1994).

In other cases, as occurs with the pepino virus (PepMV), in tomato, the resistance or tolerance, is due to a higher vigour of the rootstock (higher rate of sap flow) (Escudero *et al.,* 2003).

4. Rootstocks for solanaceae

4.1. Tomato and Aubergine

The compatibility between different species of Solanaceae is reflected in the following table:

Table 2. Compatibility between Solanaceae species

Species	Tomato	Pepper	Aubergine	Nicotiana xanthi	Datura stramonium	Solanum torvum	S.integri-folium	S.Stramoni florum	S.Sessi florum
Tomato	++++	+	++++	+++	+++	++	+++	+++	+
Aubergine	++++	+	++++	++	+++	++++	++++	++	+
Pepper	+	++++	+	+	+	+	+	+	+

Table 3. Tomato and aubergine rootstocks commercialised in Spain

	K	V	F 0.1	Fr	N	Ps.	Tm
L.esculentum x L. hirsutum							
Aegis	R	R	R	R	R		R
AR 9704	R	R	R	R	R		R
Beaufort (De Ruiter)	R	R	R	R	R		R
Maxifort (De Ruiter)	R	R	R	R	R		R
Multifort (De Ruiter)	R	R	R0,1,2	R	R		R
Unifort (De Ruiter)	R	R	R	R	R		R
Brigeor (Gautier)	R	R	R	R	R		R
King Kong (Rijk Zwaan)	R	R	R	R	R		R
Big Power (Rijk Zwaan)	R	R	R	R	R		R
Emperador (Rijk Zwaan)	R	R	R	R	R		R
Jedi (Rijk Zwaan)	R	R	R	R	R		R
Eldorado (Enza Zaden)	R	R	R	R	R		R
Triton (Western Seeds)	R	R	R	R	R		R
Monstro (Western Seeds)	R	R	R	R	R		R
He-Man (Syngenta)	R	R	R	R	R		R
He-Wolf (Syngenta)	R	R	R	R	R		R
AR 97009 (R. Arnedo)	R	R	R	R	R		R
Huron (Intersemillas)	R	R	R	R	R		R
Javato (Intersemillas)	R	R	R	R	R		R
Jedi	R	R	R	R	R		R
(L. esculentum x L.hirsutun) x L. esculentum							
Resistar (Hazera)	++	R	R	R	R		R
L. esculentum x L. pimpinellifolium							
Spirit (Nunhems)	?	R	R	R	R		R
L. esculentum							
TM 00089 (Sakata)		R	R	R	R	++	
Suketto (Agriset)		R	R	R	R	++	R
Monstro (Western Seed)							
Solanum melongena							
Java (Takii)		R					
Red Scorpion (Takii)							
Solanum torvum							
Torvum vigor (Ramiro Arnedo)		R	R	?	R	R	

Up to a few years ago, tomato grafting in Spain was not very widespread. Most of the hybrids have a range of resistances to soil-borne diseases (V,F,N), which made grafting unnecessary. The spectacular spread of grafting has been due to the importance that "collapse" has achieved. The plants grafted onto interspecific hybrids (*L. esculentum x L. hirsutum*) are more vigorous than non grafted tomato plants, and have born the effects of the same, suffering hardly any damage while the non grafted plants have been devastated. The vigour of the grafted plants also permits the use of a lower planting density, as well as better standing the adverse climatic conditions, mainly the cold.

Interspecific hybrids of *Lycopersicum esculentum x L.hirsutum* are mainly used for tomato. Some interspecific hybrids are made up of L. pimpinellifolium lines, which are resistant or tolerant to *Ralstonia* (Obrero *et al.,*1971).

Also *Lycopersicum esculentum* is used, although they are not as vigorous as the interspecific hybrids, but they have a certain tolerance to bacteriosis and can be used in tomatoes in countries where this type of disease is significant.

In aubergines, rootstocks of the types mentioned before are used. They are recommended in not very fertile soils or soils contaminated by *Pyrenochaeta*. In very fertile soils which are contaminated by *Pyrenochaeta* it is preferable to use *S. torvum* (Ginoux *et al.,* 1991). This rootstock has been used as Solanaceae rootstock in Japan (Kubota *et al.,* 2008). It is resistant to *Fusarium, Verticillium*, nematodes and *Ralstonia solanacearum*. The resistance to nematodes is maintained at high soil temperatures. Another rootstock used for this crop is *Solanum sysimbrifolium,* (Porcelli *et al.,*1990). This rootstock is tolerant to *Ralstonia* and nematodes, although it is not as resistant to *Verticillium* (Bletsos *et al.,* 2003).

The *Solanum melongena* rootstocks are exclusively used for aubergines to which they give more vigour.

4.2. Pepper

Pepper is only compatible with other Capsicum. It displays a bad affinity with other Solanaceae and even with some taxa of its same species. The current pepper rootstocks have a good behaviour regarding "soil fatigue" and root asphyxia, showing a good vigour.

Table 4. Rootstocks to be used in pepper crops specifying resistances

Rootstocks	Vegetal Material	Company	TMV	ToMV	PVY	BPeMV	Pc	Ma	Mi	Mj
Atlante	¿?	Ramiro Arnedo	R, 0							
Brutus	F1	Gautier		R, 0,1	R, 0, 2		IR	R	R	R
Tresor	F1	Nunhems	R	R	IR, 0, 1	R				
WS 2004	F1	Western		R,0			R		IR	

5. Rootstocks for cucurbits

5.1. Watermelon

The rootstocks commonly used belong to one of the following groups:

- **Cucurbita Hybrids. (*C. maxima x C. moschata*).** They are the most used. These rootstocks are also tolerant to Monosporascus, to melon necrotic spot virus MNSV, to *Verticillium*, *Pythium* and nematodes, although they are affected by the last ones under high inoculum density conditions. The interspecific hybrids transmit much vigour to the watermelon grafted onto them.
- **Cucurbita sp.** Also, other Cucurbita species and varieties can be used as watermelon rootstocks, like winter squash (*C. moschata*) as well as other *C. máxima* varieties. All of them are resistant to Fon, but its affinity with watermelon must be checked prior to use, because not all varieties and lines are compatible with it.
- ***Lagenaria siceraria***. Not very commonly used in Spain, although probably the most used watermelon rootstock in Eastern countries. It is resistant to Fon, although susceptible to *F.oxysporum* f.sp *lagenariae* (resistant lines are being selected) and *Monosporascus*. It is less affected by nematodes than the interspecific hybrids. Usually, this roostock is less vigorous than Cucurbita hybrids and less productive. Fruits have a smaller size. In both cases, differences are small.

- ***Citrullus lanatus***. These are some lines of *Citrullus lanatus* or *Citrullus citroides* or hybrids between both of them (Heo 2000, quoted by Lee 2003). They are resistant to the three known races of Fon. Their main advantage is that they are more resistant to nematodes (Meloidogyne) than the other rootstocks. They are not resistant to Monosporascus and to MNSV. The watermelon plants grafted on to this rootstock give better quality fruits than those grafted onto Cucurbita.

Table 5. Resistance of the different rootstocks used in cucurbits

	Resistance of the different rootstocks						
	Fon	**Fom**	**Phom**	**Mon**	**V.d**	**MNSV**	**Nem**
Cucurbita híbrida	+++	+++	++	+++	+++	+++	+
Lagenaria siceraria	+++		?	?	-	?	+
Citrullus sp.	+++		-	-	-	-	+++
Cucurbita moschata	+++	+++	?	?	?	?	++
Cucumis melo		+++	-	-	-	-	-

Table 6. Rootstocks for cucurbits

Rootstocks	Company	Species	Recommended crops by company
Azman RZ	Rijk		
Zwaan	C.maxima x C. moschata	Cucumber	
Watermelon			
Brava	Séminis		
Petoseed	"	Watermelon	
Carnivor	Syngenta	"	----
Ercole	Nunhens	"	----
F-33	Fitó	"	Watermelon
F-90	Fitó	"	Watermelon
Ferro RZ	Rijk		
Zwaan	"	Melon	
Watermelon			
Hércules	Ramiro		
Arnedo	"	Watermelon	
Melon			
Patrón	Clause	"	Watermelon
RS-841	Séminis		
Royal Sluis	"	Watermelon	
Melon			
Shintosa			
camelforce	Nunhems	"	Melon
Shintoza	Intersemillas	"	Watermelon
Squash nº 3	Sakata	"	Watermelon
Strong Tosa	Syngenta	"	Melon
Watermelon			
Titán	Ramiro Arnedo	"	Watermelon
Ulises	Ramiro		
Arnedo	"	Watermelon	
Melon			
Accent	Nunhems	Cucumis melo	Melon
Robusta	Intersemillas	Citrullus sp	Watermelon
T-158	Takii	¿	Watermelon

5.2. Melon

The most widespread rootstocks are the Cucurbit hybrids. With varieties of the Galia and Cantaloup melon types and also alficoz cucumber (*C. melo* var *flexuosus*), the affinity of these rootstocks is usually good, although with other types of melon, (yellow, honey and Spanish green), sometimes, a bulge or rot is produced in the higher part of the graft, which ends up with the death of a stem or the whole plant. These rootstocks provide good vigour and, usually, a yield increase, although it is not shown as clear as in the case of watermelon.

Cucumis melo. When the Fusarium (Fom) is the problem of melon, rootstock of this species can be used, as it is resistant to the Fom races 0, 1 and 2 and tolerant to the race 1-2. Although there are melon varieties with resistance to MNSV, this is not comparable with that of Cucurbita hibrida.

Other cucurbits are mentioned as possible melon rootstocks, such as *C. ficifolius, C. metuliferus, C.zeyheri,* and *C.anguria* (Buzi *et al*., 2004). *C. metuliferus*, resistance or tolerance to *Meloidogyne incognita* is especially interesting (Sigüenza *et al.,* 2005).

Photo 10. Incompatibility of the "Spanish green" melon type onto hybrid of Cucurbita

Photo 11. Incompatibility of the "honey" melon type onto the hybrid of Cucurbita

5.3. Cucumber

Cucumber grafting is spreading quickly. Interspecific hybrids of *C. máxima* x *C. moschata* and *C. ficifolia* are used as rootstocks, which are similar to those used in watermelon and melon crops. These rootstocks are resistant to *F.o. radicis-cucumerinum* (Pavlou, 2002).

Grafting on *C. ficifolia* permits farming in soil contaminated by *Phomopsis sclerotioides*, while non-grafted plants are severely affected (Dufour and Taillens, 1994).

Sycios angulatus behaves similarly to the interspecific hybrids (*C.maxima x C. moschata*) or to *Cucurbita ficifolia* (Lee *et al.,* 1994) and also, is resistant to nematodes.

6. Grafting, transplanting and planting density

When a grafted plant is transplanted, one must be careful not to cover the grafting area, as well as making sure there is a good contact between root ball and soil, to avoid the risk of *franqueamiento*. In early plantations outdoors, it is recommended to use small tunnels to keep high relative humidity and to avoid graft breakage due to wind (Miguel, 1994). When planting on padded soils in warm seasons, special attention must be paid to the size of the hole made on the plastic, with the purpose of avoiding a chimney effect if the size of this is reduced too much (Koren, 2003), (Ricárdez *et al.*, 2006). Some days after transplanting, the new sprouts of rootstock that may have been produced shall be removed.

The main problem of using grafted plants is their cost. The adjustment of the planting density is essential for an optimisation of this crop technique. Trials made by the Research Group AGR 200 of the University of Almería, within the framework of the Methyl Bromide Alternatives project promoted by ONUDI throughout the past four years in different countries from North and Central America, have concluded that densities of 50-60 %, compared with those made in different places using non grafted plants, have increased yield and kept quality in different watermelon, melon and tomato cultivars. Miguel (1993) assesses, for grafted melons and watermelons, a decrease of 30-40 % in density compared with non grafted plants and a 20 % specifically for Spanish melons. In this trial, the grafted plant, in any of its densities, showed a significantly higher yield and bigger size fruits than non grafted plants.

7. Conclusion

Grafting on resistant rootstocks is a resource that permits, in many occasions, to face up to soil pathogens in an effective and ecological way, neither contaminating the product nor the environment. Grafting must not be used solely; but combined with other techniques intended for the same purpose, following a good agronomic practice. When grafting is used, it is convenient to use additional strategies to reduce the level of inoculum in the soil (Davis *et al.,* 2008).

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