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Grafts of Crops on Wild Relatives as Base of an Integrated Pest Management: The Tomato *Solanum lycopersicum* as Example

Hipolito Cortez-Madrigal
Centro Interdisciplinario de Investigación
para el Desarrollo Integral Regional-Instituto
Politécnico Nacional, Jiquilpan, Michoacán
México

1. Introduction

After the potato, the most cultivated vegetable in the world is the tomato *Solanum lycopersicum* L. In 2009, the global area harvested was 4,393,045 ha, with one production of 152 956 115 ton. Mexico ranked 10 th place with 99, 088 ha and a production of 2,591,400 tons (FAO, 2011).

There is consensus that the origin of the tomato is South America, where is the greatest diversity of related species (wild relatives) (Peralta et al., 2005), but is also accepted that domestication of tomato occurred in Mexico (Rick & Holle, 1990; Hoyt, 1992, Perez et al., 1997). Consequently in this country, the tomato, also called "jitomate", is considered one of the basic components of Mexican cuisine. Additionally, the name "tomate" comes from the Nahuatl language of Mexico (Rick & Holle, 1990; Perez et al., 1997). After corn the tomato is the crop that has had greater genetic manipulation (Perez et al., 1997), but focused on the standpoint of productivity. It has been documented that there is an inverse correlation between the degree of domestication (productivity) of plants and damage by pests and diseases (Coley et al., 1985; Rosenthal & Dirzo, 1997); so that resistance to pests and diseases in wild relatives is higher than in native varieties of crops and these in turn show greater tolerance than hybrid modern varieties.

The tomato is one of the crops with the highest number of pests, with approximately 17 phytophagous insects. The whitefly *Bemisia tabaci* Gennadius, 1889 (Hemiptera-Sternorryncha: Aleyrodidae) and the psyllid *Bactericera* (= *Paratrioza*) *cockerelli* (Sulc, 1909) (Hemiptera-Sternorryncha: Psyllidae) are two of the most important pests (King & Saunders, 1984; Liu & Trumble, 2005; Morales et al., 2005). The conventional way of dealing with pest problems is basically through organo-synthetic pesticides, strategy that causes serious problems to the environment and human health. An alternative method is the plant resistance to pests and diseases (Kogan, 1990) and the main source of germplasm for crop improvement are the wild relatives (Hoyt, 1992; Perez et al., 1997). Thus, different species of Solanum that develop in the center of the origin of the tomato have been widely used in crop improvement by hybridization (Simons & Gur, 2005; Casteel et al., 2006; Restrepo et al., 2008); however, the conventional hybridization between tomato and its wild relatives is not always possible (Perez et al., 1997; Peralta et al., 2005); then, various desirable traits of wild plants cannot be transferred by this technique. In this regard, the grafting technique is an alternative well documented in crop improvement (Lee, 1994; Kubota et al., 2008).

2. Grafts and their use in the pest and diseases management

Grafting is a technique by which two or more plants are joined, forming a single plant; the basal part is called "rootstock" and the superior "scion". This technique has been used since ancient times to transfer desirable characteristics of one plant (rootstock) to another (scion) (Yamakawa, 1982; Lee, 1994; Poincelot, 2004; Kubota et al., 2008). Exist several reasons for using grafts. Many plants are difficult to propagate by other techniques; desirable varieties with poor root development are candidates for grafted on strong rootstock (Poincelot, 2004). Furthermore, the use of grafts may also induce tolerance to adverse environmental factors such as salinity (Martinez-Ballesta et al., 2008), drought (Pire et al., 2007) and adverse temperatures (Venema et al., 2008), among others. The grafts also tend to produce stronger plants and yielding (Khah et al., 2006). In addition, grafted plants induce better quality of fruits (Martinez-Ballesta et al., 2008; Godoy et al., 2009). However, one of the principal uses of grafts is to induce resistance or tolerance to pests and diseases, such as nematodes and soil fungi (Lee, 1994; Kubota et al., 2008).

The first documented case of resistance of grafts to insects was the control of grape Phylloxera *Dactulosphaira vitifoliae* (Fitch) in the United States. The susceptible European grapes scion grafted onto resistant American wild grapes, provided the total control of the pest (Kogan, 1990). Since then, the resistance to pest continues (Granett et al., 1987), demonstrating the sustainability of that pest management strategy.

2.1 Grafts in herbaceous plants

Grafting in herbaceous plants has been known since the nineteenth century. Japan and Korea were the first countries to develop grafting vegetables. In Europe grafting is commonly practiced (Yamakawa, 1982; Lee, 1994; Kubota et al., 2008). However, in the Americas its use in plant breeding has only recently received attention (Red & Riveros, 2001; Kubota et al., 2008; Godoy et al., 2009; Garcia-Rodriguez et al., 2010). Perhaps, one reason is because the American agricultural areas are of greater extent than those of Japan, Korea and Europe; for example, the United States is the country with one of the lowest production of grafts (Kubota et al. 2008). The aim of grafts is to induce resistance to biotic and abiotic factors, including pests and diseases, but also to improve the quantity and quality of fruits (Lee, 1994; Cañizares & Goto, 1998; Dorais et al. 2008; Kubota, 2008). Protected vegetable production without crop rotation as control measure, has led the increase of pests and diseases that are a real problem for this type of agriculture. The main alternative for nematode and disease control was the use of fumigants such as methyl bromide, but with the recent ban on its use in the Montreal protocol, the graft in vegetables is seen as a major strategy in the pest and diseases management; and in general, to transfer valuable traits to the crops (Lee, 1994; Gonzalez et al., 2008; Kubota et al., 2008; Martinez-Ballesta et al., 2008).

In recent years, the grafting has aroused as a technique of great interest in vegetable crops such as cucumber, melon, watermelon, peppers, eggplant and tomato. The grafting has been used to induce resistance to fungal diseases (Alconera et al., 1988; Bletsas et al., 2003; Garcia-

Rodriguez et al., 2010) and bacterial (Nakahara et al., 2004; Coutinho et al., 2006), and to the nematodes *Meloidogyne javanica* Chitwood, 1949, *M. incognita* Kofoid and White, 1919 and *M. arenaria* Roberts and Thomason, 1989 (Heteroderidae) (Williamson, 1998; Sigüenza et al., 2005; Verdejo-Lucas & Sorribas, 2008).

Grafts have been performed on rootstock of local varieties with low productivity but high resistance to pests and diseases. Different species of Cucurbita have been used as a rootstock for melon and watermelon grafts (Yamakawa, 1982; Cohen et al., 2005; Sigüenza et al., 2005; Kubota et al., 2008) and *Capsicum* landraces for chili (Garcia -Rodriguez et al., 2010). In other cases, rootstock have been obtained from resistant hybrids, such as watermelon rootstock from hybrids of *Cucumis maxima* x *Cucumis moschata* (Lee, 1994), or hybrids of *Lycopersicon hirsutum* x *L. esculentum* for rootstock in tomato (Yamakawa, 1982). However, the main source of resistant rootstocks are wild plants, mainly so-called "crop relatives" (Yamakawa, 1982; Alconera et al., 1988; Gonzalez et al., 2008; Kubota et al., 2008; Venema et al., 2008).

3. Importance of crop wild relatives

During its evolution, the wild relatives of crops have developed many features that have enabled them to survive in extreme conditions; for example, on the shores of the Galapagos Islands there is a wild relative of tomato that has provided genes to the cultivated tomato conferring high tolerance salinity, so the plants can be irrigated with one-third seawater (Hoyt, 1992). Also, the main source of resistance it is found in wild plants, and close relatives of crops have been the most exploited in plant breeding (Hoyt, 1992; Ramanatha Rao & Hodgkin, 2002).

No wonder that the main source for grafts has been the rootstock of wild plants, which besides other characteristics have become resistant to pests and soil diseases, such as fungi and nematodes (Yamakawa, 1982; Alconera et al., 1988; Gonzalez et al., 2008; Kubota et al., 2008; Venema et al., 2008). Thus, has been common to graft watermelon on *Lagenaria siceraria* (Yamakawa, 1982; Lee, 1994; Yetis & Sari, 2003); the eggplant, on their wild relatives *Solanum integrifolium* and *Solanum turvum* (Yamakawa, 1982; Lee, 1994; Bletsas et al., 2003); cucumber, on *Cucurbita ficifolia*, *Sicyos angulatus* (Lee, 1994) and *Cucumis metuliferus* (Sigüenza et al., 2005); melons, on *Cucurbita* spp., *C. moschata*; tomato on *L. pimpinellifolium* and *L. hirsutum* (Lee, 1994); there are reports of tomato grafts onto the weed *Datura stramonium* L. that were practiced for many years in the Southeastern of The United States (Kubota et al., 2008).

When wild plants are used, besides to be resistant to pests and diseases or have some other desirable characteristic, it is advisable to know the effect of the rootstock on the fruit quality. For example, it has been documented that some rootstocks may influence the nutritional characteristics of fruits (Martinez-Ballesta et al., 2008) and even get translocation of toxic compounds into the scion, as happened with the first tomato grafts in wild solanum *D. stramonium* (Kubota et al., 2008). It has recently been documented that the effect of the rootstock towards the graft can even up the genetic level (Zhang et al., 2008).

3.1 Tomato wild relatives

As a native American plant, tomato has a wide diversity of wild relatives in that continent, among those mentioned: *S. cheesmaniae* (L. Riley) Fosberg, *S. pimpinellifolium* L., *S.*

chmielewskii (CM Rick, Kesicki, Fobes & M. Holle) D. M. Spooner, G. J. Anderson & R. K. Jansen, *S. neorickii* (CM Rick, Kesicki, Fobes & M. Holle) D. M. Sponner. G. J. Anderson & R. K. Jansen (= *L. parviflorum*), *S. habrochaites* S. Knapp & D. M. Spooner (= *L. hirsutum*), *S. chilense* (Dunal) Reiche, *S. peruvianum* L., *S. penelli* Correll and *S. lycopersicum* var. *cerasiforme* L. (Esquinas & Nuez, 1995; Peralta et al., 2005).

Such is the importance of wild relatives of tomato that modern varieties would not exist without the wild relatives; characteristics such as resistance to cold or extreme conditions and resistance to pests and diseases have been transferred from wild relatives to cultivated plants (Hoyt, 1992, Perez et al., 1997). Then, the knowledge and conservation of crop wild relatives is of utmost importance in global food production (Hoyt, 1992; Eigenbrode & Trumble, 1993; Perez et al., 1997).

Unfortunately, “modern” agricultural practices as the use of herbicides and other chemicals have led to a gradual loss of biological diversity and populations of wild relatives of crops (such tomatoes) have been drastically depleted (Hoyt, 1992; Vargas, 2008; Alvarez-Hernandez, 2009a).

It is accepted that the closest ancestor of cultivated tomato is *S. lycopersicum* var. *cerasiforme* D. M. Spooner, G. J. Anderson and R. K. Jansen, 1993 (Esquinas & Nuez, 1995; Peralta et al., 2005), grows in a wide variety of habitat from 0 to 3 300 meters above sea level (Sanchez-Peña et al ., 2006; Vargas, 2008; Alvarez-Hernandez et al., 2009a;), characterized by having round fruits with diameters ranging from 1 to 2.5 cm (Martinez, 1979; Rick et al., 1990). In some states of the Center-Western Mexico, the wild tomato is known as "tinguaraque" (Martinez, 1979). So in this paper frequently we use that name. Since 2005 we have developed studies about the tolerance of tinguaraque to phytophagous insects and its potential as rootstock in grafts with cultivated tomato. The research questions included:

- Which is the incidence of phytophagous insects on tinguaraque?
- Which is the preference of *Bactericera cockerelli* for tomato, tinguaraque and grafts from both?
- How is the incidence of insects’ pest on tomato, tinguaraque and grafts from both under field conditions?
- Which characteristics present tomato fruits grafted on tinguaraque?
- Which is the response of tomato grafts on tinguaraque at different nutrimental handling systems?

4. The tinguaraque (*Solanum lycopersicum* var. *cerasiforme*) in Mexico

4.1 Importance and distribution

In Mexico, the tinguaraque is widely distributed in ecological reserves and associated crop fields where it eventually tends to become a weed (Perez et al., 1997; Sanchez-Peña et al., 2006). It features a high capacity for climate adaptation, it was found from 7-2 000 meters above sea level, with annual rainfall of 495-1 591 mm, annual mean minimum temperature from 7.1-21.6 °C, 22.6-38.4 °C mean annual maximum temperature, and between 15.8 and 28.1 °C mean annual temperature (Vargas, 2008). Sanchez-Peña et al. (2006) reported populations of wild tomato at altitudes from 12 to 1 104 masl on the Northest of Mexico.

In warm regions (<300 masl) populations of wild tomato are reduced and are associated with species that provide shade; in temperate regions these plants protect them from the cold (Vargas, 2008). Because of its creeping growth habit-climbing, it is common to found the wild tomato associated with different plants; for example, many plants were climbing among the thorny branches of the "acacia" (*Acacia* spp.) scattered among grass and weeds. The dispersion of its branches is a survival strategy to pests and herbivores (Alvarez-Hernandez et al., 2009a).

Partial collections in the Mexican state of Michoacan, showed that its distribution includes altitudes from 314 to 1 550 masl, maximum annual temperatures ranged from 26.9 to 35.2 at minimum of 11.7 °C to 26.9 °C; annual precipitation of 751 mm to 1 866 mm and with varying levels of soil fertility; similarly pH values ranged from 6.8 to 8.5 (Alvarez-Hernandez et al., 2009a, Table 1). The pH values obtained exceeding the normal limits for the development of cultivated plants whose optimal value is between 6.0 and 7.5 (Michel et al., 1998); by contrast, the cultivated tomato is considered tolerant to the acidity values of 5.5- 7.5 and higher values are limiting (Valadez, 1998).

This has allowed that tinguaraque have populations with different characteristics in response to biotic and abiotic factors of mortality according to the conditions where it develops. However, it also indicated that urban growth and agricultural production techniques, as use of herbicides, are the main factors influencing the loss of tinguaraque diversity; there are even regions where it is known there were populations of tinguaraque; however, nowadays farmers do not know about its existence (Vargas, 2008; Alvarez-Hernandez et al., 2009a).

Physicochemical variables	**Sampling sites**			
	Apatzingán	**Acahuato**	**Los Reyes**	**Jiquilpan**
pH	8.3	6.8	8.5	7.6
Sand (%)	19.7	26.0	24.0	15.9
Silt (%)	40.3	35.0	29.0	34.3
Clay (%)	40.0	39.0	47.0	49.8
Organic matter(%)	3.0	4.9	2.5	7.7
Total nitrogen (%)	0.12	0.2	0.10	0.3
Phosphorus mg/kg	17.1	17.4	16.6	15.7
Potassium meq/100 g	3.3	3.3	0.4	1.1

Table 1. Physical and chemical characteristics of soils obtained from sites with wild tomato populations in three regions of Michoacán, Mexico (Alvarez-Hernandez et al., 2009a).

In Michoacán state, populations of wild tomato were found restricted to habitat where agricultural impacts are minor, such as roadsides, areas with thorny plants, waterways, river banks, among others (Alvarez-Hernandez et al., 2009a).

4.2 Morphological and physiological characteristics of tinguaraque

Based on the fruit size, Alvarez-Hernandez et al. (2009a) identified two groups of tinguaraque in Center-western Mexico: Small-fruited (1.05 to 1.22 cm of polar diameter and 1.10 to 1.25 cm of equatorial diameter) and large-fruited (2.12 to 2.23 cm of polar diameter and 2.41 to 2.55 of equatorial diameter); the cultivated tomato fruit has an average of 10 cm (Valadez, 1998; Muñoz, 2009) and its weight ranges from 5 to 500 g (Chamarro, 1995). The fruit size is closely related to the number of seeds and the number of locules (Muñoz, 2009), variable that seems to be interesting to evaluate. One characteristic of wild tomatoes is to present a smaller number of locules than those grown; commercial cultivars are multilocular type (Valadez, 1998), while the wild have two locules (Rick et al., 1990; Alvarez-Hernandez et al., 2009a).

Another important feature in wild tomato species is the highest density of trichomes compared to cultivated varieties. Sanchez-Peña et al. (2006) compared the density of trichomes on *S. habrochaites* (C-360), *S. lycopersicum* var. *cerasiforme Vs* the commercial variety Rio Grande. They found that the density of trichomes was higher in the first species, followed by *S. lycopersicum* var. *cerasiforme*, and the cultivar had the lowest density of trichomes. In this regard, it is known that trichomes are one of the main factors that induce resistance to pests in tomato (Eigenbrode & Trumble, 1993; Wagner et al., 2004).

Wild plants as tinguaraque generally have a slower germination compared to cultivated varieties. In this regard, Alvarez-Hernandez et al. (2009a) found a tendency for greater speed and uniformity in germination of commercial tomato "Rio grande" compared to the germination of wild populations of tinguaraque; the time when 50% of seeds germinated ranged from 2.8 (2.5-3.0) to 10.6 (8.6-15.7) days in tinguaraque, whereas in the commercial cultivar was 4.4 (4.0-4.8) days. In general, the germination rate in large-fruited tinguaraques was similar to the cultivated tomato, suggesting a direct relationship between speeds of germination and fruit size (Table 2).

The observed differences in germination tinguaraques suggests two things: first, that the different climatic conditions where these populations grow and the time spent as wild plants could be determinants of the germination speed (Alvarez-Hernandez et al., 2009a); for example, tropical species of plants usually germinate faster than temperate species (Meletti & Bruckner, 2001); second, similar germination recorded in tinguaraques large fruited and the cultivar suggest that these tinguaraques perhaps have less time as wild plants, and even yet are handled by humans (Alvarez-Hernandez et al., 2009a). It is currently accepted the hypothesis that the var. *cerasiforme* is a wild tomato escaped from cultivation (Esquinas & Nuez, 1995; Peralta et al., 2005).

Population	**GT50 * (days)**	**Fiducial limits (days)**	**Prob. Chi. Sq.**
Little Apatzingan	8.5	7.4-10.6	0.0001
Big Apatzingan	4.9	4.6-5.2	0.0001
Acahuato	6.4	5.2-9.3	0.0001
Los Reyes	2.7	2.5-3.0	0.0001
Jiquilpan	10.6	8.6-15.6	0.0001
Tabasco (big)	4.9	4.7-5.2	0.0001
Cv. Rió Grande	4.3	4.0-4.7	0.0001

* Germination Time of 50% of seeds.

Table 2. Germination rate of six wild tomato ecotypes collected in Michoacán and Tabasco, Mex. and cv. Rio Grande (Alvarez-Hernandez et al., 2009a).

A practical use of knowledge of the germination rate could be used to improve crops by grafting. Having this base of time and germination percentage, it is possible to standardize the development stages of compatible species, but with different rates of development, as occurs in wild and cultivated tomato, the first slower in its development.

4.3 Phytophagous insects associated with tinguaraque

Few studies have been documented about the entomo-fauna of S. l. var. *cerasciforme*, but it is mentioned that wild tomato can tolerate high incidence of pests and diseases (Hoyt, 1992; Eigenbrode & Trumble, 1993; Nakahara et al., 2004; Sanchez-Peña et al., 2006).

After one year of sampling in three different climatic regions of Michoacan, Mexico (Apatzingan, Los Reyes and Jiquilpan), five groups of insects were recorded: whitefly (Hem: Aleyrodidae), aphids (Hem: Aphididae), leaf miners (Dip: Agromyzidae), psyllids (Hem: Psyllidae), horn and fruit worms (Lepidoptera), and fleahopper (Col: Chrysomelidae) (Alvarez-Hernandez et al., 2009a; Table 3). In general, those groups include some of the main pests of cultivated tomato (King & Saunders, 1984).

The incidence of phytophagous insects observed in tinguaraque was low and consequently damage to plants was also low; for example, only few specimens of hornworm *Manduca* spp were registered. Similarly, about three larval specimens of chrysomelids (Chrysomelinae) were recorded. In the three collection sites, the bug *Cyrtopeltis notata* (Distant) (Hemiptera: Myridae) was the most abundant phytophagous insect recorded on tinguaraque; Due the frequency and damage of this species, it could be considered a potential pest of tinguaraque (Table 3). Moreover, not all pests were equally distributed in the regions; so, the tomato psyllid *B.* (=*Paratrioza*) *cockerelli* was only registered in one región (Jiquilpan). *B. cockerelli* is considered a major pest of the cultivated tomato (Liu & Trumble, 2005). Therefore, it is important to consider populations of tinguaraque with longer coevolution with the pest, could be probably more resistant to it.

Order: Family	Species
Hemiptera: Aleyrodidae	*Bemisia tabaci y Trialeurodes vaporariorum*
Hemiptera: Aphididae	Species complex
Hemiptera: Myridae	*Cyrtopeltis notata* Distant
Hemiptera: Psyllidae	*Bactericera cockerelli* Sulc.
Diptera: Agromyzidae	*Lyriomiza sativae* Blanchard y *L. trifoli* Burgess
Lepidoptera: Sphingidae	*Manduca sp*.
Lepidoptera: Noctuidae	*Heliothis sp*.
Coleoptera: Chrysomelidae	*Epitrix sp*.
Coleoptera: Chrysomelidae	*Chrysomelinae*

Table 3. Major groups of phytophagous insects registered in wild populations of tinguaraque collected in Michoacán, Mex. (Alvarez-Hernandez et al., 2009a).

Diversity in that wild populations of *S. lycopersicum* develops, marks its importance as a resource adaptable to different climatic conditions prevailing in Mexico (Vargas, 2008). The wide variability of wild ecotypes of *S. lycopersicum* var. *cerasiforme* (Dunal), presumably with resistance to certain pests and diseases is an aspect useful for crop improvement. Previous reports have pointed out resistance of wild tomato to various tomato pests, including: *Liriomyza* sp., armyworm *Spodoptera exigua* (Hiibner), bugs complex (Hemiptera) (Eigenbrode & Trumble, 1993) and whitefly *B. tabaci* (Sanchez-Peña et al., 2006); resistance to early blight *Rhyzoctonia solani*, late blight *Phythophthora infestans* (Pérez et al., 1997) and potato rot *Ralstonia* (= *Pseudomonas*) *solanacearum* (Nakaho et al., 2004) has been documented. However, genetic improvement through hybridization is usually slow, expensive and eventually there are barriers to conventional hybridization (Perez et al., 1997; Poincelot, 2004). Grafts on wild relatives or plants resistant to pests and diseases have proven to be an important tool for crop improvement (Poincelot, 2004; Kubota et al., 2008). Therefore, it was interesting to know the response of tinguaraque and its grafts with cultivated tomato to the incidence of the insect pests.

5. Incidence of pests in grafts of tomato with tinguaraque

5.1 The tomato psyllid *Bactericera cockerelli*

Few are the documented studies about grafting in vegetables with native species in Mexico (Garcia-Rodriguez et al., 2010), therefore the wealth of germplasm has been wasted, and in some cases at risk of disappearing. Therefore, the study was aimed to evaluate the resistance of grafting of tomato in its wild relative *S. lycopersicum* var. *cerasiforme* of the region of Jiquilpan, with emphasis on the tomato psyllid *B.* (= *Paratrioza*) *cockerelli (*Hem: Psyllidae). This insect is one of the major pest of tomato, with losses of up to 85%. Although often ineffective, its control is based on the chemical method; however, other control strategies have been suggested, including plant resistance (Liu & Trumble, 2005; Casteel et al., 2006).

In field conditions we evaluated the incidence of phytophagous insects on *S. lycopersicum* var. *cerasiforme*, ecotype Jiquilpan. Results showed low incidence of insect pests on tinguaraque and particularly *B. cockerelli* was one of the species with lower incidence. In order to confirm this observation, we established an experiment including tomato, tinguaraque, and graft of both. In laboratory conditions, plants were confronted with a known number of adults of *B. cockerelli* and its preference for each plant was registered. The incidence of pests was also considered in field conditions.

Consistently, the insect preferred tomato, graft and tinguaraque in that order. When treatments were exposed individually, the highest incidence occurred in tomato psyllid (16.0 ± 10.1) and lowest in tinguaraque (7.5 ± 3.0) and graft (8.3 ± 6.8), in that order. When the three treatments were presented simultaneously, the preference of adult psyllids was 22.8 times higher in tomato than tinguaraque, and three times higher than for grafts (Table 4). This was confirmed in field trials where the largest number of adults, nymphs and oviposition was recorded in the cultivated tomato, and the lower number in tinguaraque. The graft showed intermediate number, but without differences with the tinguaraque (Table 5).

Treatment	Incidence (%)	
	Individual bioassay	Multiple bioassay
	Mean (%) ± DS[1]	Media (%) ± DS[1]
Tomato	16.00 ± 10.1 a	15.03 ± 9.26 a
Graft	8.33 ± 6.89 b	4.99 ± 2.57 b
Tinguaraque	7.50 ± 3.03 b	0.66 ± 0.71 c
N	**6**	**8**

[1] Means ± standard deviation, with the same letter into column, are not statistically different (Tukey, 0,05).

Table 4. Incidence of *Bactericera cockerelli* (adults) on tomato, tinguaraque and graft of both when they were exposed in individual and multiples bioassays (Cortez-Madrigal, 2010).

5.2 Incidence of other pests

The main groups of phytophagous insects recorded were: aphid species complex (Hemiptera: Aphididae), *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae); complex bugs (Hemiptera), highlighting the species *C. notata* (Myridae) and the leaf miners *Liriomyza* spp. (Diptera: Agromyzidae). Although was observed a trend towards a higher incidence of insects in cultivated tomato, statistically differences only were registered for miners and aphids, where the highest and lowest incidence was for tomato (3.9±3.18) and tinguaraque (0.68±0.79). The graft showed an intermediate incidence (2.18±2.16). The highest and lowest incidence of aphids was in tomato and tinguaraque in that order (0.758 ± 0.98 y 0.237 ± 0.36). The graft showed an intermediate relation respect to tomato and tinguaraque, but there were no statistical differences between them (Table 6).

Treatment	Adults	Eggs	Nymphs	N
Tomato	1.04 ± 1.01 a	0.46 ± 0.43 a	1.13 ± 0.97 a	12
Graft	0.27 ± 0.46 b	0.12 ± 0.09 b	0.27 ± 0.24 b	12
Tinguaraque	0.35 ± 0.71 b	0.10 ± 0.10 b	0.21 ± 0.19 b	12

Mean ± standard deviation after log (x+1) transformation followed by the same letter within columns do not differ statistically (Tukey, 0.05). N= number of repetitions.

Table 5. Incidence of *Bactericerca cockerelli* on tomato, tinguaraque and graft of both in field conditions from Jiquilpan, Michoacan, Mexico (Cortez-Madrigal, 2010).

Treatment	Leaf miner	Aphids	N
Tomato	3.9 ± 3.18 a	0.758 ± 0.98 a	12
Graft	2.18 ± 2.16 b	0.316 ± 0.35 ab	12
Tinguaraque	0.68 ± 0.79 c	0.237 ± 0.36 b	12

Means ± standard deviation after log (x+1) transformation followed by the same letter within columns do not differ statistically (Tukey, 0.05).

Table 6. Average incidence per plant of leaf miner and aphids on tomato, tinguaraque and graft of both under field conditions in Jiquilpan, Michoacan, Mexico. Year 2007 Cortez-Madrigal, 2010.

Although there were no statistical differences in the incidence of whitefly, graphically shows the trend of lower incidence in tinguaraque; contrary, tomato, followed by graft showed the highest incidence of the pest. Only in Hemiptera complex the incidence was similar in tomato, tinguaraque and grafting (Fig. 1).

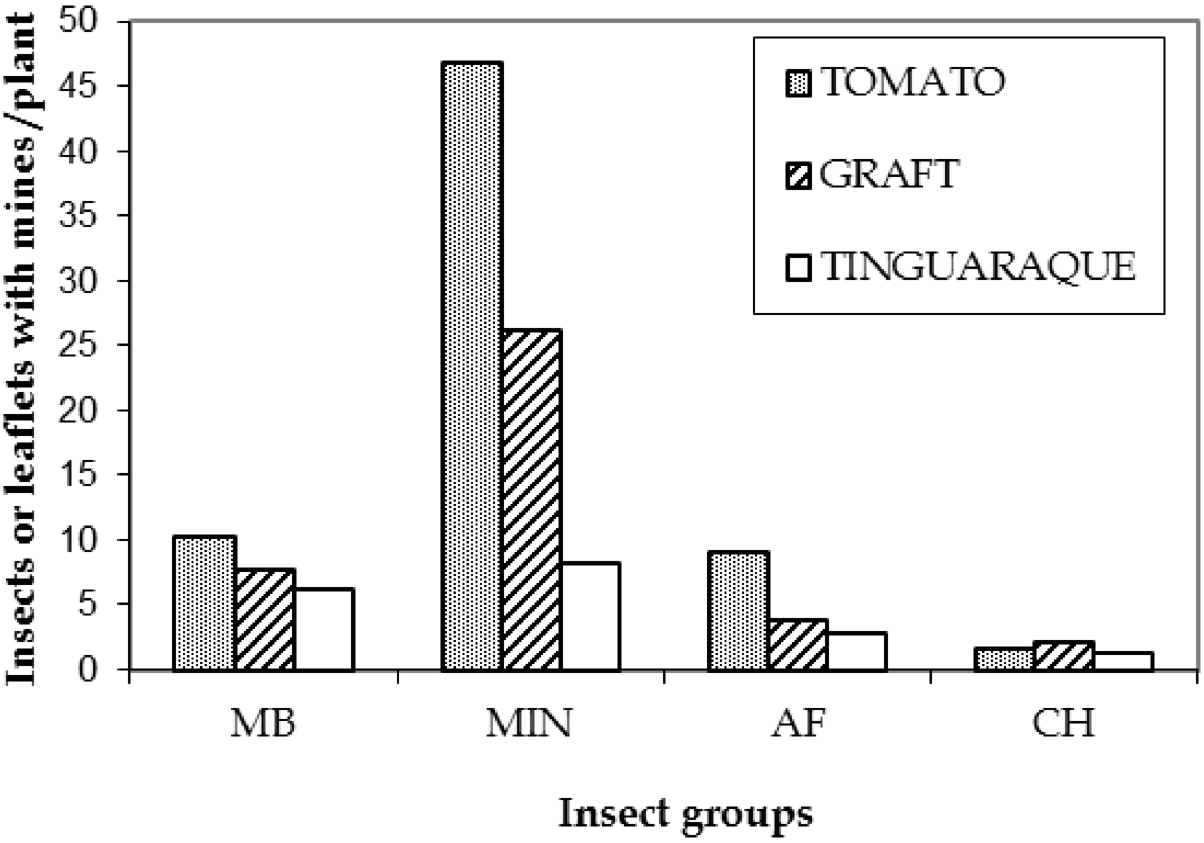

Fig. 1. Incidence of whitefly (MB), leaf miner (MIN), aphids (AF) and bugs (CH) in tomato, grafting and tinguaraque (Cortez-Madrigal, 2010).

5.3 Incidence of pests in tomato grafted with different ecotypes of tinguaraque

Given the wide variability of conditions where tinguaraque grows in Mexico, we considered convenient to evaluate different ecotypes, from temperate regions to warm places. In accordance with the above-mentioned, the study aimed to evaluate the incidence of phytophagous insects in tomato grafting on various ecotypes of *S. lycopersicum* var. *cerasiforme* native from Michoacan, Mexico. The experiment was established in the region of Apatzingan Valley at an altitude of 300 masl. The climate is a Bs1 (h ') w (W) corresponding to the semi-dry warm climate with summer rains (Garcia, 1988). The mean temperature, annual minimum and maximum are 28, 20 and 37.7 °C, respectively. The average rainfall, minimum and maximum is 834, 500 and 972.8 mm, in that order. The type of soil was a vertisol pelico (INEGI, 1983).

Thirteen treatments were established: five wild ecotypes of *S. lycopersicum* var. *cerasiforme* natives from Michoacan (GAp, ChAp, Ac, LR and Jiq) and one from Tabasco (Tab); six grafts of tomato cv. Toro onto tinguaraque (I-GAP ... I-Tab), and the cv. Toro as control (Tom).

From November 17, 2007 to February 16, 2008, weekly samplings were implemented in Ciudad Morelos, Municipality of Paracuaro, Michoacan, Mexico.

The main species of insects registered were: whitefly *B. tabaci*, psyllid *B. cockerelli* and the aphid *Aphis gossypii*. Results showed a wide variability of responses from the tinguaraque ecotypes and its grafts, generally with lower incidence of pests compared to those registered on tomato without grafting. Again, tolerance of tinguaraque and its grafts toward diverse insect pests was registered (*B. cockerelli* and *B. tabaci*).

For Whitefly adults only one tendency to lower incidence on grafting was registered. The lowest incidence was in the graft I-GAp (13.14 ± 7.18), compared with 17.6 ± 10.4 in the ungrafted tomato. The graft with tinguaraque Tabasco (larger fruit) showed an incidence similar to that of the cultivated tomato (Table 7).

The incidence of whitefly nymphs showed significant differences (p = 0.0001). Grafts showed an intermediate response, where stood the treatments I-GAp and I-ChAp (which were native tinguaraque), with significant differences respect to the cultivar (Table 7). Regarding *B. cockerelli*, treatments with lower incidence of adults were tinguaraques small fruit and grafts, where I-ChAp and I-GAp were the best. Conversely, the highest incidence of the adults occurred in the tinguaraques large fruit (Tab and GAp), and the commercial variety (Tom). Regarding the incidence of nymphs of *B. cockerelli*, there were no differences between treatments.

For the aphids, the lowest incidence occurred in the graft GAp (1.52 ± 1.22) along with tinguaraques small fruit; the highest incidence occurred in the commercial cultivar (4.82 ± 5.22) without differences with tinguaraques large-fruit. Most of the grafts showed an intermediate response (Table 7).

Treatment	**Insect species**				
	B. tabaci		***B. cockerelli***		**Aphididae**
	Adults	**Nymphs**	**Adults**	**leaflets with eggs**	
Tom	17.6±10.4* abc	9.7±8.5 a	2.2±1.9 ab	1.6±1.2 ab	4.8±5.2 a
I-LR	15.6±8.4 abc	7.5±5.9 abcd	1.7±1.7 bcd	1.2±1.3 ab	2.3±2.5 bcd
I-Jiq	15.3±8.5 abc	7.5±5.8 abc	1.6±1.6 abcd	1.3±1.3 ab	2.3±2.3 bcd
I-ChAp	14.5±7.9 bc	6.6±5.6 bcd	1.3±1.1 d	1.0±0.9 ab	2.2±2.1 bcd
I-Ac	15.7±8 abc	7.1±5.6 abcd	1.6±1.6 abcd	1.1±1.0 ab	2.2±2.7 bcd
I-GAp	13.1±7.1 c	5.8±4.6 bcd	1.3±1.1 cd	0.9±0.7 b	1.5±1.2 d
I-Tab	16.0±9.3 abc	7.8±6.8 abc	1.9±2.2 abcd	1.3±1.6 ab	2.8±3.2 bcd

*Means ± standard deviation after log (x+1) transformation followed by the same letter within columns do not differ statistically (Tukey, 0.05).

Table 7. Incidence of phytophagous insects in a cultivated variety of tomato and their grafts with different ecotypes of tinguaraque *S. lycopersicum* var. *cerasiforme* (Alvarez-Hernandez et al., 2009b).

According to a multivariate analysis of the incidence of pests, new groups of plants were formed; in the case of *B. cockerelli* (adults and eggs) five groups were formed: one consisting of the cultivated tomato (Tom) and tinguaraque G-Ap, very close to the group formed by the tinguaraque Tabasco (Tab), corresponding all of large fruit. Another group was formed by grafting and tinguaraques Jiquilpan (Jiq) and Los Reyes (LR). The tinguaraque "chico apatzingan" (ChAp) as a single group. Finally, the graft Tabasco (I-Tab) and tinguaraque Acahuato (Ac) formed another group (Fig. 2).

The commercial variety and tinguaraques large fruit were usually the ones that had the highest incidence of pests. Tinguaraques Small-fruit showed lower incidence and in turn, the grafts showed an intermediate trend. This coincides with what is stated about the incidence of pests and the degree of domestication of plants (Coley et al. 1985; Rosenthal & Dirzo, 1997). Modern varieties of tomatoes have been genetically manipulated more than tinguaraques, and within these, there may be some that are already handled by humans, as in the case of tinguaraque Tabasco, which is marketed in their origin region.

5.4 Development studies

Recent unpublished studies on the incidence of whitefly (*B. tabaci* and *T. vaporariorim*) on grafts of tomato with tinguaraque under different nutritional levels, the results confirm previous studies (Alvarez-Hernandez et al., 2009a, b; Cortez -Madrigal, 2010;) in the sense that grafts are less affected than ungrafted tomato. Additionally, the production of grafted plants was similar to that of ungrafted plants (Table 8).

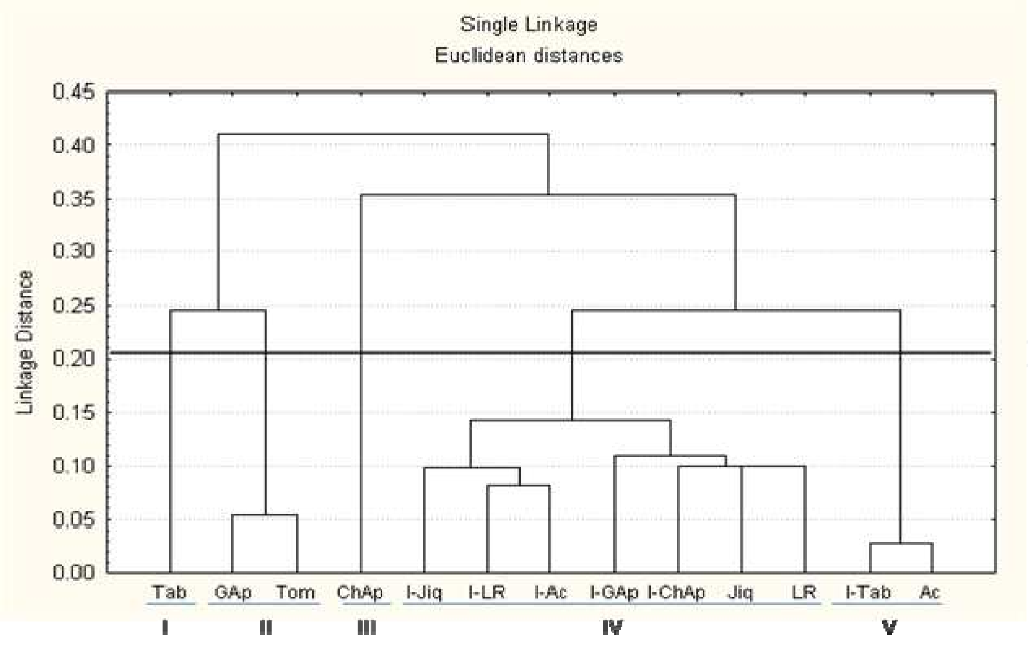

Fig. 2. Dendrogram showing the formation of groups of tomato, tinguaraques and grafts of boths based on the incidence of *B. cockerelli*. Apatzingan, Michoacan, Mexico. 2007. Tom = tomato, Tab = Tinguaraque Tabasco, Jiq = Tinguaraque Jiquilpan, LR = Tinguaraque Los Reyes, Ac = Tinguaraque Acahuato, GAp = Big tinguaraque Apatzingan, ChAp = Small tinguaraque Apatzingan, I = Graft.

Treatment	Means[1]± STD
Ungrafted with compost (U-C)	58.84±38.5 A
Ungrafted with fertilizer (T-F)	28.39±20.6 AB
Grafted withaout fertilizer (G-WF)	16.46±10.8 BC
Fertilized graft (G-F)	7.63±5.3 C
Ungrafted or fertilized (U-WF)	6.76±3.2 C

[1] Mean ± standard deviation after log transformation (x +1) followed by the same letter do not differ statistically (Tukey, 0.05).

Table 8. Incidence of whitefly *B. tabaci* and *T. vaporariorum* on tomato grafted and ungrafted under different nutritional levels. Jiquilpan, Mich. 2010.

6. Tomato fruit quality grafted on tinguaraque

An important aspect to consider is to know the quality of fruit grafting; studies such: size and production, color, acidity, soluble solids and sugars in the fruit should be included. Alvarez-Hernandez (2009) characterized biochemically fruit quality of grafts of tomato on tinguaraque and concluded that fruits of the grafts were not different from the fruit without grafting (Table 9).

Treatment	**Variable**			
	pH	**Soluble solids (ºBrix)**	**Humidity (%)**	**Density**
ChAp	5.07*±0.05	6.0*±0.0	90.73	1.48
GAp	5.02±0.05	6.0±0.0	91.43	7.06
Ac	5.35±0.1	7.75±0.5	89.94	1.31
LR	4.77± 0.05	7.75±0.5	89.05	1.02
Jiq	4.87± 0.05	7.5±0.57	90.13	0.94
Tab	5.37± 0.05	5.25±0.5	88.39	7.17
I-ChAp	4.67± 0.05	6.25±0.5	97.37	10.67
I-GAp	4.55± 0.05	6.75±0.5	93.99	9.75
I-Ac	4.45± 0.05	6.0±0.0	97.44	7.95
I-LR	4.45± 0.05	6.5±0.57	96.52	9.80
I-Jiq	4.5±0.0	5.5±0.57	96.41	10.67
I-Tab	4.5±0.00	6.75±0.5	97.44	10.50
Tom	4.52±0.09	7.0±0.0	94.28	9.79

*Means ± standar deviation.

Table 9. Physical and chemical characteristics of tomato fruits, tinguaraque and graft of both. Parácuaro, Michoacán, Mexico (Alvarez-Hernandez, 2009). Tinguaraques: ChAp, GAp, Ac, LR, Jiq y Tab; grafts: I-ChAp…I-Tab; commercial variety: Tom.

Previous reports indicate resistance of *S. lycopersicum* var. *cerasiforme* to various pest and diseases of tomato (Eigenbrode & Trumble, 1993; Perez et al., 1997; Nakahara et al., 2004; Sanchez-Peña et al., 2006). The results of our studies agree with those mentioned by Eigenbrode & Trumble (1993) in the sense that wild tomato has resistance to leaf miner *Liryomiza* spp. more does not match the resistance indicated by these authors for the complex of Hemiptera. In our case, resistance of tinguaraque was clearer to *Liryomiza* spp., *B. cockerelli* and aphids (Aphididae), but not for the bugs complex, consisting mostly of the species *C. notata* (Hem: Myridae).

The differences in the incidence of pests found between tinguaraques small fruit and large fruit is probably related to the density of trichomes. In this regard, Sanchez-Peña et al. (2006) found higher densities of trichomes on wild tomatoes than in the cultivated variety, but there were also significant differences between populations tinguaraque. It is known that the main mechanisms of pest resistance in tomato depends on the density and type of trichomes, which have distinguished seven types, including glandular and non-glandular trichomes (Simmons & Gurr, 2005); the first are involved in production of allelochemicals as acilsugars (Mutschler et al., 1996; De Resende et al., 2008), zingiberene (Freitas et al., 2002) and decanonas (Muigai et al., 2002), substances that cause insect repellency or mortality. Similarly, non-glandular trichomes play a role as physical barriers in the establishment and development of some insects (Eigenbrode & Trumble, 1993; Wagner et al., 2004).

Trichomes, mainly glandular, are generally more abundant in wild than in cultivated species (Sanchez-Peña et al., 2006; Simmons et al., 2006), and in some cases there has been a strong correlation between incidence of phytophagous insects and density of trichomes (Simmons et al., 2004; Alba et al., 2009). However, in other cases the production of allelochemicals has not clearly correlated with the density of trichomes, suggesting that independent mechanisms of resistance are involved (Nombela et al., 2000; Muigai et al., 2002), where the pH of the leaf would be a major factor; has been documented, for example that *B. tabaci* prefers cotton sheets with a pH of 6-7.25 (Berlinger, 1983).

The fact that the grafted material have shown lower incidence of pests than the commercial cultivar, suggests that the graft favored tolerance to recorded tomato pests. The incidence of insects was three times lower in grafts than in ungrafted tomato; however, mechanisms involved in this tolerance are unknown. Might think that secondary substances anti-herbivores are synthesized in the wild rootstock and from there translocated into the susceptible scion; however, some grafts with the lower incidence of pests were formed by wild rootstock obtained from tinguaraques in which the highest incidence of insects occurred. Therefore, the tolerance of grafts to insects could be multifactorial, as has been noted by other authors (Muigai et al., 2002).

The resistance of the tomato wild relatives has been used to obtain plants with resistance to pests and disease, mainly through hybridization (Casteel et al., 2006; Restrepo et al., 2008), slower than the development of grafts. Although the use of grafts in vegetables is a common practice in much of Asia and Europe (Lee, 2003; Nakahara et al., 2004; Verdejo-Lucas and Sorribas, 2008), in American countries has been little explored and less commonly used to transfer resistance to pests and diseases (Gonzalez et al., 2008; Garcia-Rodriguez et al., 2010).

Usually, grafts have been directed to pathogen and soil pests resistance (Lee, 1994; Kubota et al., 2008) where is located the rootstock resistant and little has been documented about its effect on the aerial pests. Although some scientist written disclosure mentioned the grafts resistance to aerial pests, do not show experimental evidence that support his claim (Kubota & Viteri, 2007). The results obtained by us show that through grafts were formed new groups of plants with a lower incidence of pests than on commercial variety without grafting; even, some of the best treatments were grafts.

Insects as Paratrioza and whiteflies are major pests of cultivated tomatoes and other vegetables, so these results may be important utility in the production of these crops, initially at the greenhouse and gardens level. However, other pest as the hornworm *Manduca* spp., bollworm *Heliothis* spp. and pinworm *Keiferia lycopersicella* (Walsingham) must be included in future studies.

7. Conclusions

The grafting of cultivated tomato on the wild tomato *S. lycopersicum* var. *cerasiforme* has potential in the management of foliar pests such as *B. cockerelli*, *Liriomyza* spp. complex of aphids (Aphididae) and apparently to *B. tabaci*. The grafting technique developed by us is simple and inexpensive, so it can be implemented by any producer. Its use is primarily focused on low-income farmers who grow tomatoes in small areas, although it is feasible to use in greenhouse crops with greater use of inputs.

Although by mean of graft was not reduced completely insect damage, it is important to consider that his action was on several species, some considered key pests of tomato. We understand the use of grafting as a tool of integrated pest management. Under this view, other control strategies should be evaluated, where ecological methods should be prioritized. For example, micoinsecticides, yellow traps and even low-toxicity insecticides, among others. For countries considered origin center of crops, such as Mexico, to conserve and use wild relatives of crops as source of resistance to pests and diseases should be a priority. In Mexico grow many wild relatives of crops, including *S. lycopersicum* var. *cerasiforme*. Growing adjacent to agricultural fields and modern farming techniques, such as herbicide application, threaten its permanence. The development of grafts in wild relatives can give them more value and contribute to the conservation of these species.

The fruits of tomato grafted on tinguaraque were not modified, at least in their basic biochemical characteristics. Since the tinguaraque is edible, it is feasible to think is not necessary to develop toxicological studies of grafted fruit. However, the organoleptic quality whether it should be investigated. Some compounds of interest could be found in greater concentration in tinguaraque and be transferred by grafting to tomato. This would be a plus to the fruits of the grafts.

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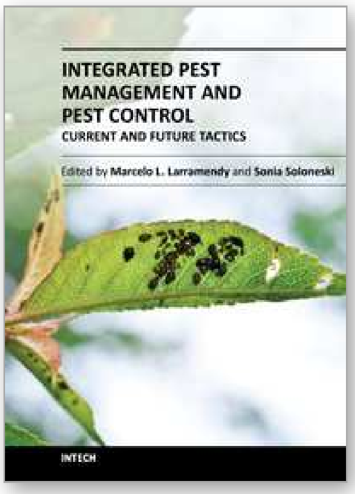

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821