Freezing Tolerance of *Opuntia* spp.

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ABSTRACT

Frost is a serious abiotic stress affecting crops in the Chihuahuan desert. An unusually severe frost occurred 11-14 December 1997, and caused serious injuries in *Opuntia* spp. commercial orchards for nochtli, tuna, or prickly-pear fruit production in Mexico's State of Zacatecas and at the Centro Regional Universitario of the Universidad Autónoma Chapingo germplasm collection. An evaluation of resistance to that frost was carried out in a collection of 73 varieties (five plants each) of *Opuntia* spp. in the germplasm bank. The percentage of dead cladodes (%DC) was measured in individual plants that were used as the experimental unit. Means and standard deviations (μ, $\pm\sigma$) of the %DC are discussed. The varieties "Duraznillo" and "Tapón Aguanoso" were not affected by the freeze. The "Amarilla CE", "Amarilla SM", "Morada SM", "Camuezo" and "Zarquita" varieties were only slightly affected (0%DC to 10%DC) by the freeze, so these varieties, in combination with the "Tapón Aguanoso" and "Duraznillo" varieties, are the only varieties able to withstand cold temperatures without serious injury. In particular, the COPENA varieties were quite susceptible to freezing weather. These findings will be useful for Opuntia on crop research and genetic improvement programs.

Key Words: Freezing injury; cold hardiness; Cacti; *Opuntia* varieties; Nochtli (cactus pears in the Nahuatl language), tuna or prickly-pear fruit

INTRODUCTION

One of the most serious abiotic stresses that plants growing in the Chihuahuan desert face is freezing weather. Overwintered crops must survive subzero temperatures unharmed in order to have good early growth in the spring. In the southern part of the Chihuahuan desert, however, various frost sensitive perennial orchard crops are occasionally severely damaged by unusually hard winters or even by transient overnight radiation frosts.

In Zacatecas state, México, 13901 ha of *Opuntia* spp were planted prior to 1992 (Flores-Valdez and Gallegos-Vázquez, 1994) for fruit production (prickly pear in English, tuna in Spanish, and nochtli in Nahuatl or Mexica) to exploit the region's natural resources, i.e., soil and climate. The Opuntia fresh fruit matures from July to October. Rain-fed fruit production is a good option as in 1992, growers obtained a higher income from Opuntia (2604 Mexican pesos or US$260) as compared to 122 pesos and 300 pesos for corn and beans, respectively (Flores-Valdez and Gallegos-Vázquez, 1994). The main *Opuntia* spp. "cultivated varieties" in Zacatecas State were "Burrona", "Amarilla", "Pico Chulo", Blanca San José", "Fafayuca", "Rojo Pelón", and "Cristalina" with 40%, 22%, 14%, 5%, 4%, and 3% of the planted area, respectively (Flores-Valdez and Gallegos-Vázquez, 1994).

During 11 through 14 December 1997, freezing weather occurred and caused serious damage to *Opuntia* spp. commercial orchards and in the Universidad Autónoma Chapingo (UACh) germplasm bank of the Centro Regional Universitario Centro Norte (CRUCeN). This freeze had a minimum temperature of about –11°C (Figure 1) as measured by a nearby meteorological station. Except for two short periods, there were two and one-half days with temperatures below zero (Figure 1). Growers did not know how to protect the cacti because Opuntia spp. have been under cultivation for only 20 to 25 years in this location.

Although various methods to evaluate damage to freezing-weather growth have been studied, visual browning tests have been the most widely used by cold-hardiness researchers (Kang et al., 1998) because these procedures are simple and quickly determine the viability of plant material. After plant tissues are subjected to controlled freezing temperatures, they are either dissected or permitted to see if they produce new growth. If no new growth occurs, the tissues are rated as dead. Smaller samples of various tissues such as stem sections, flower buds, and root systems can be dissected. Tissues that are brown or do not look similar to that of the nonfrozen tissues are rated as dead. If roots are studied, those that are brown, appear wet, and slough off when pulled slightly are also rated as dead. One disadvantage, however, is that a great deal of time is required to dissect and score all the samples. However, a grower could dissect plant tissues in the field with a sharp knife to determine the percentage of damage. This technique allowed Kang et al. (1998) to evaluate the freeze damage to persimmons and other *Diospyros* spp. at the orchard of Kyoto University, Japan, after a freeze on 13 and 14 April 1996. Stergios and Howell (1973) believe that these tests are accurate in determining death and can be used as controls for other techniques.

Ishikawa and Gusta (1996) reported that cladodes of *Opuntia fragilis* (a species native to southern Saskatchewan, Canada) taken from a plant growing in the middle of the normal growing season did not tolerate an overnight freeze of –3.5°C. However, terminal cladodes collected in midwinter tolerated a slow freeze to –50°C without any apparent signs of injury, and in comparison with *O. fragilis*, the terminal cladodes of *O. polyacantha* collected in midwinter were less freezing tolerant (–40°C) (Ishikawa and Gusta, 1996).

Griffiths (1915) discovered various cold-hardy spineless varieties useful for forage and was the first to report effects of freeze damage to Opuntia. Martinez (1969) was the first to report Opuntia fruit varieties that had improved cold hardiness. From 300,000 seedlings established in 1961, only 45 survived a –16°C freeze in 1962, and Martinez (1969) grew 31 of these to maturity and evaluated their fruit production. Borrego-Escalente et al. (1990) reported various clones that also had survived –16°C in Saltillo, Mexico. A research group in southern Texas, reported various evaluations of Opuntia for improved cold hardiness and fruit production (Russell and Felker, 1987; Gregory et al., 1993; Parish and Felker, 1997; Wang et al., 1998). Nobel (1990) measured the uptake of the red dye neutral red (which is taken up only by live tissues) after cladode segments were subjected to various temperatures for one hour in a ethylene glycol/water bath and found good correlations between this laboratory test and freeze hardiness as evaluated in field trials. Nobel (1995) also found that injections of large amounts of glucose into cladodes greatly stimulated the cold tolerance of both *O. ficus-indica* and *O. humifusa* and suggested that the concentration of water in the extracellular spaces controlled freeze damage in Opuntias.

In this communication we report an evaluation of resistance by 73 *Opuntia* spp. collections established at the germplasm bank of the Centro Regional Universitario Centro Norte (CRUCeN) of the Universidad Autónoma Chapingo (UACh), located near Zacatecas city, México to a severe freeze during 11 through 14 December 1997. The main aim was to rank the collections as to low, intermediate, and high resistance to freezing weather.

MATERIALS AND METHODS

The CRUCeN-UACh *Opuntia* spp. germplasm bank

The CRUCeN-UACh *Opuntia* spp. germplasm bank was located at El Orito village, Zacatecas, México, 4 km southwest from Zacatecas City, at 22° 44' 42" N longitude and 102° 36' 24" W latitude. The climate of the area is dry, with scarce precipitation events throughout the year and with a total average rainfall of 440 mm per year. The major rain period is from July to October, during which 70% of rainfall occurs.

The soil was a calcareous litosol. After various collection trips in the central-north region of México, five or seven cladodes of each variety were planted in 1987–1988, 1991–1992; 1993–1994, or 1994–1995) (Gallegos-Vázquez et al., 1996). The germplasm bank was treated routinely with pesticides to control fungal diseases. The 73 collections and their sites of origin are listed in Table 1.

We prefer to call these collections varieties because they are not sufficiently distinctive to be considered separate and independent *Opuntia* species (Harrington and Durrel, 1957). However, these taxonomic varieties should not to be confused with the horticultural "varieties" of cultivated plants (Harrington and Durrel, 1957). Although the COPENA clones developed by the late Dr. Facundo Barrientos, and "Fafayuca", "Burrona", "Cristalina", "Amarilla", "Pico Chulo", "Blanca San José", "Rojo Pelón", and a few other varieties have been referred to as horticultural varieties by local people, these "varieties" were not a result of a serious breeding program; therefore, they should be treated simply as cultivated native species. These 73 *Opuntia* spp. varieties will be referred to by their common names because they are remarkably descriptive and are the only names known to most people.

Data

The plants were naturally subjected to the 11 through 14 December 1997 freeze that had a minimum temperature of about –11°C (Figure 1). This slow freezing and thawing caused serious injuries to both cladodes and whole plants, depending on the variety. After a week we were able to detect injured cladodes visually as the color turned from green to brown. The injured cladodes were counted and pruned from the plant with a knife. All of these pruned cladodes turned brown and were rated as dead cladodes. From previous determinations of total number of cladodes for five plants before the freeze, we determined the percentage of dead cladodes for the 73 varieties of the first and second dates of collection (1987–1988 and 1991–1992) using equation 1.

$$\%DC=\{(TNC)(NDC)\}/100 \quad (1)$$

where (%DC) is the percentage of dead cladodes, (NDC) is the number of dead cladodes, and (TNC) is the total number of cladodes for the five plants per variety in the field.

RESULTS AND DISCUSSION

Commonly, sine and log transformations are used to analyze the percentage of leaf damage (e.g., Fuller et al., 1989) to avoid nonadditivity, variance heterogeneity, and non-normality. Therefore, we examined log transformations of the variety means (μ) and their standard deviations (σ) of the %DC. The %DC ranged from zero to 100 and there was no evidence for a strong linear relationship between the log μ and log σ (Figure 2). Thus, a simple power transformation is not appropiate for such data. While distribution-free, nonparametric methods should be considered (Fernandez, 1992), we simply used the means and standard deviations(μ, $\pm\sigma$) (Figure 3).

The *Opuntia* spp. varieties differed in their inherent frost tolerance, as can be seen in Figure 3. Moreover, the %DC mean values and standard errors show inflections larger than the error bars when plotted from the more to the less cold-hardy varieties. It means that the difference in frost tolerance among the varieties was relatively large by considering that few did not have dead cladodes (i.e., "Duraznillo *O. leucotricha* " and "Tapón Aguanoso *O. robusta*"), most had a variable %DC, and, in a few accessions, the entire plants died (e.g., "C. C1" and "Amarillo 2"). We do not feel that the age of the plant was associated with the overall freeze-damage rating because all the plants were mature and more than 5 years old. However, the youngest cladodes were affected first in all varieties except "Duraznillo" and "Tapón Aguanoso". This indicates a fairly wide base from which to select for improved frost tolerance by conventional procedures. A relevant aspect is that the unharmed varieties "Duraznillo" and "Tapón Aguanoso" are native collections and they are growing wild in their natural environment near the experimental site.

These two freeze-resistant varieties had some distinguishing morphological features. The "Duraznillo" variety had a large number of relatively small spines that may create a protective microenvironment covering each cladode. The "Tapón Aguanoso" variety is particularly characterized by its vertical cladodes and because it is known that cladode orientation and inclination (Cano-Santana et al., 1992) influence cladode temperature, this morphological feature may be of assistance in minimizing freeze damage. We believe these two varieites should be used in a plant-breeding program to try to confer these cold-tolerant genes to other improved varieties that are cultivated currently in Zacatecas state.

The large variation in %DC of the varieties "Solferino", "Cascarona", "Azucarona", "Apastillada", "Rojo", "T. Loca", "Morada 3", "Alfajayucan", "B. Palo Alteña", "C. 5", "Naranja", and "Tapón Mayo" (Figure 2) may be masking some of the intrinsic intervarietal differences. This great variability is not to be unexpected because various authors have shown that *Opuntia* is highly polymorphic. Wang et al. (1998) found that *O. lindheimerii*, which is native to northern Mexico and is unharmed by temperatures of –20ºC can be hybridized to commercial *O. ficus-indica* fruit varieties. These authors further suggested that back crosses to this native species could be used to confer freeze hardiness to the commercial fruit varieties.

All the spineless materials, especially the spineless COPENA clones, which are usually identified as *Opuntia ficus-indica*, were among the most frost sensitive of the *Opuntia*s. This is in agreement with Nobel (1990), Russell and Felker, (1997), Gregory et al. (1993), Parish and Felker (1997), and Nobel (1995) who generally reported the ranking of cold hardiness as being:

O. cochinillifera < *O. ficus-indica* < *O.robusta* < *O. streptacantha* < *O .lindheimerii* < *O. humifusa*.

One plausible mechanism for the resistance of freeze hardiness in *Opuntia*s would be freeze avoidance via supercooling. However, supercooling is a nonequilibrium condition and has been demonstrated to not be the mechanism by which freezing injury is avoided by cacti (Nobel, 1988). The exact ability of the cladodes from the unharmed plants and varieties is unknown. We believe it would be useful to determine the freezing points by exotherm detection as reported by Pierquet and Stusshnoff (1979), Flinn and Ashworth (1994), Kang et al. (1997, 1998), and Fuller and Wisniewski (1998) for other genera.

CONCLUSIONS

Frost tolerance varies considerably among taxonomic varieties of platyopuntias. The "Tapón Aguanoso" and "Duraznillo" varieties were not affected by the freeze. The next most resistant varieties were "Amarilla CE", "Amarilla SM", "Morada SM", "Camuezo" and "Zarquita" with 0%DC to 10%DC. The "Pabellón", "Blanca Palo Alto", "Liso Forrajero", "Blanca Cerro" and "Cristalina 1" varieties had an intermediate level of resistance to frost (25%DC to 70%DC) while the most affected varieties with

80%DC to 100%DC were the COPENA clones ("C. 15", "C. 2", "C. CE2", "C. F1", "C. V1", "C. 16", "C. V2" and "C. C1"), "Fafayuca", "Tuna Morada 1", "Charola Tardía", "Sangre Toro", "Bola Masa", "Burrona 2", "Amarillo 1", "Tuna Morada", "Colorada", "Rubí Reyna" and "Amarillo 2". The "Apastillada", "Rojo", and "Alfajayucan" varieties showed a great intervarietal variation of resistance to frost. We hypothesize that the striking difference in cold hardiness between amarilla and amarillo varieties (from most to least cold hardy) is attributable to the site of collection. The frost sensitive varieties "Amarilla CE", "Amarilla SM", and "Amarilla SE" were from Chapingo, Mexico, and collected by Dr. Facundo Barrientos in the cold high plain or from Puebla state. In contrast, we collected "Amarillo 1" and "Amarillo 2" in the northern part of Mexico's central region.

The native species (*O. leucotricha* and *O. robusta*) and the Amarilla varieties from México state as the site of origin have freezing tolerance much greater than those from the north or south central region of the country.

These results indicate the importance of studying the pattern of freezing in *Opuntia* spp. Because a large number of cladodes in most of the varieties were incapable of surviving freezing, one might imagine that the only procedure of frost tolerance is by freeze avoidance via supercooling. However, supercooling is a nonequilibrium condition, but it is not the mechanism by which freezing injury is avoided by cacti (Nobel, 1988). However, the exact ability of the cladodes from the unharmed plants and varieties is unknown. This is a problem that could be solved through determining freezing points by exotherm detection as measured by Pierquet and Stusshnoff (1979), Flinn and Ashworth (1994), Kang et al. (1997, 1998), and Fuller and Wisniewski (1998) with different genera and species.

We feel it would be important to use techniques such as infrared thermography to rapidly and nondestructively measure the temperatures at various locations in the canopy of mature Opuntia plants during such freezes to determine the pattern of the damage. This can be done by using, at least, the differential thermal analysis (Kang et al., 1998), and the relative electrical (Fuller et al., 1989), and infrared thermography methods (Fuller and Wisniewski,1998) Also, it would be most helpful to use the cold hardy varieties "Duraznillo *O. leucotricha*" and "Tapón Aguanoso *O. robusta*" as parents in a breeding program to improve the freeze hardiness of the current commercial varieties in central Mexico.

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Table 1. Collections of *Opuntia* spp. Established at the Centro Regional Universitario Centro Norte of the Universidad Autónoma Chapingo Germplasm Bank and Their Sites of Origin

Common Name	Site of Origin	Specie	Common Name	Site of Origin	Specie
Copena 2	Tecamachalco, Pue.	*O. amyclaea*	Naranjón	Pinos, Zac.	*O.* spp.
Copena 3	Tecamachalco, Pue.	*O. amyclaea*	Blanca Palo Alto	Los Azulitos, Jal.	*O.* spp.
Copena 5	Tecamachalco, Pue.	*O. amyclaea*	Rosa de Castilla	Santa Rosa, Ags.	*O.* spp.
Copena 14	Tecamachalco, Pue.	*O. amyclaea*	Verdulero	Pinos, Zac.	*O.* spp.
Copena 15	Tecamachalco, Pue.	*O. amyclaea*	Camuezo	Santa Rosa, Ags.	*O.* spp.
Copena 17	Tecamachalco, Pue.	*O. amyclaea*	Moradilla 1	El Terremoto, Ags.	*O.* spp.
Copena 18	Tecamachalco, Pue.	*O. amyclaea*	Zarquita	Los Azulitos, Jal.	*O.* spp.
Burrona 1	Ojocaliente, Zac.	*O.* spp.	Memelo 1	El Terremoto, Ags.	*O.* spp.
Copena 16	Tecamachalco, Pue.	*O. amyclaea*	Manso Colorado	El Terremoto, Ags.	*O.* spp.
Amarillo	Valparaíso, Zac.	*O.* spp.	Duraznillo	Palo Alto, Ags.	*O. leucotricha*
Fafayuca	Obraje, Pinos, Zac.	*O. amyclaea*	Tapona Mayo	Palo Alto, Ags.	*O. robusta*
Charola Tardía	V. de Arriaga, SLP.	*O.* spp.	Tapón Aguanoso	Palo Alto, Ags.	*O. robusta*
Bola de masa	Obraje, Pinos, Zac.	*O.* spp.	Pachón	Montoya, Ags.	*O.* spp.
Burrona 2	Obraje, Pinos, Zac.	*O.* spp.	Solferino	La Nopalera-UACh	*O.* spp.
Cristalina 1	Obraje, Pinos, Zac.	*O.* spp.	Copena L-12	Fruticultura-CP	*O. amyclaea*
Tuna morada 1	Obraje, Pinos, Zac.	*O.* spp.	Copena Torrioja	Las Papas, Ojuelos, Jal.	*O.* spp.
Amarillo 1	Obraje, Pinos, Zac.	*O.* spp.	Apastillada	La Nopalera-UACh	*O.* spp.
Mango	Obraje, Pinos, Zac.	*O.* spp.	Azucarona	La Montesa, V. G., Zac.	*O.* spp.
Colorada	V. de Arriaga, SLP.	*O.* spp.	Caña Tierna	La Montesa, V. G., Zac.	*O.* spp.
Amarillo 2	V. de Arriaga, SLP.	*O.* spp.	Morada 3	La Montesa, V. G., Zac.	*O.* spp.
Pico Chulo 1	V. de Arriaga, SLP.	*O.* spp.	Tuna Loca	La Montesa, V. G., Zac.	*O.* spp.
Pico Chulo 2	V. de Arriaga, SLP.	*O.* spp.	Cuatroalba	La Montesa, V. G., Zac.	*O.* spp.
Cristalina 2	V. de Arriaga, SLP.	*O.* spp.	Blanca de Cerro	Los Campos, Zac.	*O.* spp.
A. Montesa	V. de Arriaga, SLP.	*O.* spp.	Cascarona	Los Campos, Zac.	*O.* spp.
Rubi Reyna	V. de Arriaga, SLP.	*O.* spp.	Rojo	La Nopalera-UACh	*O.* spp.
Cardona	S. Mateo, Valp., Zac.	*O. streptacantha*	Amarilla CE	La Nopalera-UACh	*O.* spp.
Liso forrajero	S. Mateo, Valp., Zac.	*O. ficus-indica*	Amarilla SE	La Nopalera-UACh	*O.* spp.
Pabellón	Ojocaliente, Zac.	*O. ficus-indica*	Morada SM	La Nopalera-UACh	*O.* spp.

Table 1. Collections of *Opuntia* spp. Established at the Centro Regional Universitario Centro Norte of the Universidad Autónoma Chapingo Germplasm Bank and Their Sites of Origin (continued)

Common Name	Site of Origin	Specie	Common Name	Site of Origin	Specie
Tuna Morada 2	S. Mateo, Valp., Zac.	*O.* spp.	Rojo Vigor	La Nopalera-UACh	*O.* spp.
Copena F1	Chapingo, México.	*O. ficus-indica*	Amarilla SM	La Nopalera-UACh	*O.* spp.
Copena C1	Chapingo, México.	*O. ficus-indica*	Alfajayucan	La Nopalera-UACh	*O. amyclaea*
Copena C2	Chapingo, México.	*O. ficus-indica*	Copa de Oro	S.L.P., S.L.P.	*O.* spp.
Tuna Rosa	S. Mateo, Valp., Zac.	*O.* spp.	Isabelona	Guadalupe, Zac.	*O.* spp.
Copena V1	Chapingo, México.	*O. ficus-indica*	Sangre Toro	V. de Arriaga, SLP.	*O.* spp.
Copena V2	Chapingo, México.	*O. ficus-indica*	Comicalla	O. Crucitas, Ags.	*O.* spp.
Copena Z1	Tecamachalco, Pue.	*O. amyclaea*	Sandía	Palo Alto, Ags.	*O.* spp.
Copena Z2	Tecamachalco, Pue.	*O. amyclaea*			

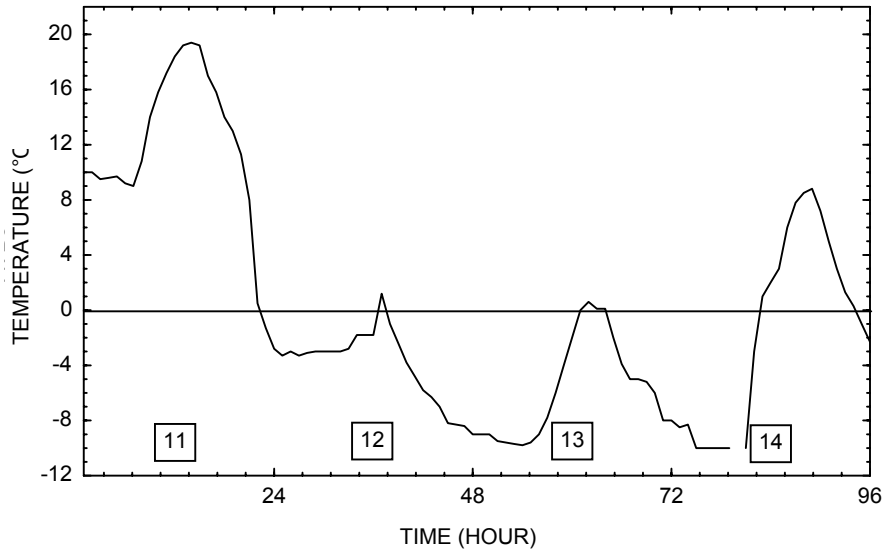

Figure 1. Air Temperature Registered from 11 to 14 December 1997 at the Guadalupe, Zac., México Meteorological Station (22° 44' 47" N, 102° 30' 23" W, and 2258 masl). (This station is located near the CRUCeN-UACh *Opuntia* spp. germplasm bank site. Days are identified inside squares on the time (X) axis. Disruption of the trace is because temperatures below –10°C are not registered by the thermograph.)

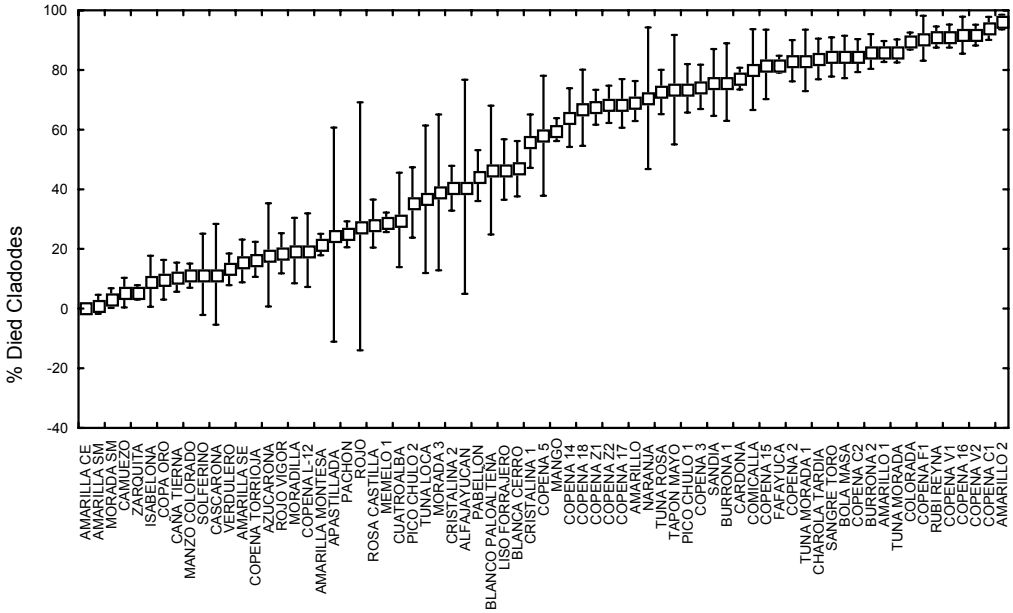

Figure 2. Plot of the Variety Log Means (μ) *versus* Log Standard Deviations (σ) of the Percentage of Dead Cladodes (%DC) for 71 *Opuntia* Taxonomic Varieties. The "Duraznillo" and "Tapón Aguanoso" varieties are not considered because of their zero values.

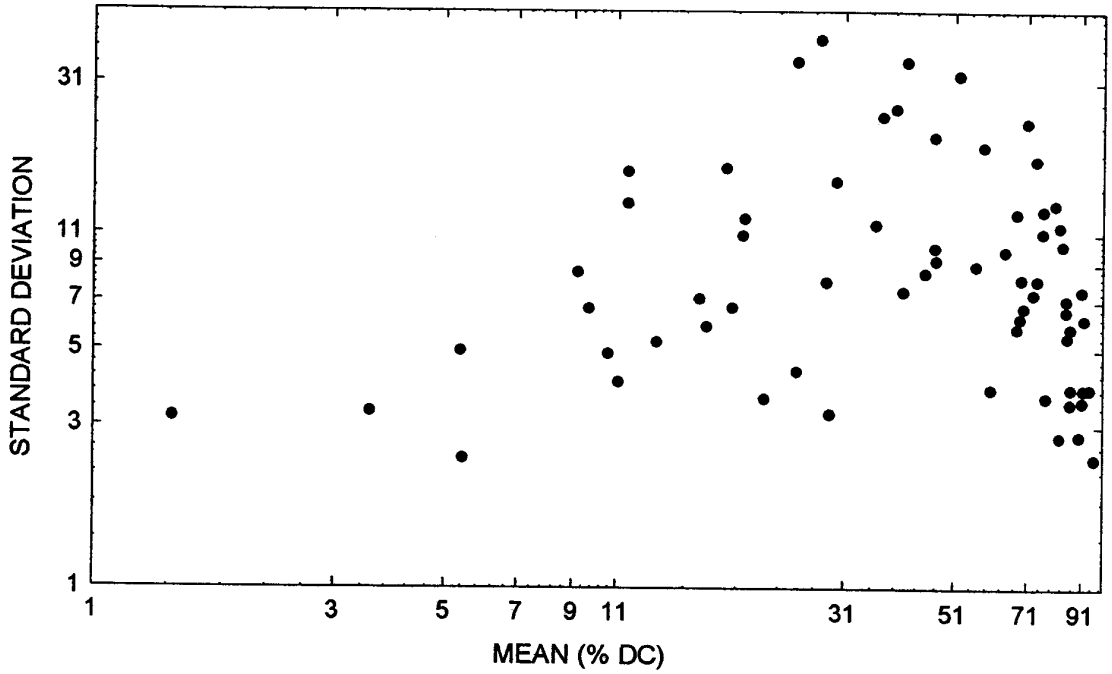

Figure 3. Scores of Injury as Mean ± Standard Deviation (mean and error bars) of Percentage of Dead Cladodes (%DC) for 71 *Opuntia* Taxonomic Varieties Exposed to the 11 through 14 December 1997 Frost at the CRUCeN-UACh Germplasm Bank. The "Duraznillo" and "Tapón Aguanoso" varieties were excluded from this graph because of their zero damage rating.