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In Vitro Production of *Vitis* spp Callus and Leaves for Protoplast Isolation

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Close source of resistance for most of the known cultivars used as scions and rootstocks of the *Euvitis* subgenera species ($2n=38$) are the cultivars of *Vitis rotundifolia* ($2n=40$). Propagation of *V. rotundifolia* by cuttings is difficult due to their poor rooting characteristic and conventional crossings between *Euvitis* and *V. rotundifolia* is complicated because of their chromosomal number difference. Somatic hybridization via protoplast provides an opportunity for gene transfer between sexually incompatible related species. *In vitro* explants usually present thinner foliar cuticle which facilitates protoplast isolation. Alternatively, the fact that a cultivar is successfully established *in vitro* might be a signal of the protoplasts totipotency. The goal of this work was to test 11 different cultivars of grapes with different desirable characteristics for their *in vitro* capability and maintenance, callogenesis, organogenesis and protoplast isolation. Different media and benzyladenine (BA) concentrations were tested. From the eleven cultivars tested, C. sauvignon, Syrah, SV 12-375, Scuppernong, Magnolia, Higgins and B. beauty were the most successful *in vitro*, completing all stages of propagation. Regenerated plants are being kept in greenhouse. Black Spanish and Herbemont callus have been kept for long period *in vitro* however plants were not regenerated. SV-12327 and Jumbo died. Cultivars showed different response to different media and BA concentrations. *V. rotundifolia* cultivars presented no rooting problems *in vitro*. Leaves were the preferred source for protoplast isolation comparing to callus and suspension cells. Protoplasts were isolated from Herbemont and C. sauvignon; Herbemont protoplast formed microcallus and are being kept in culture.

Biological Control of Giant Salvinia at Lewisville Aquatic Ecosystem and Research Facility

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Releases of the salvinia weevil were made in 2003 and 2004 in four ponds found at Lewisville Aquatic Ecosystem and Research Facility (LAERF) located in Lewisville, TX. Each of the four ponds received about 38,400 insects since the first release date. The most recent samples collected in late 2005 indicated the presence of 5 to 17 adult weevils per five salvinia plants. This data confirms the establishment of the biological control agent. Observations of the ponds show that weed populations have severely decreased due to weevil feeding damage.

Attraction of Mexican Fruit Flies to Colored Traps and Lures

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In the recent past, several studies have evaluated the attractiveness of colored multilure traps/lure combinations to capture the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae). When promising traps and lures from those trials were used in a current program for monitoring *A. ludens* in the citrus orchards of northeast Mexico, the results were very variable. Thus, we reconsidered the issue and evaluated, green and yellow multilure traps plus a clear multilure trap more alike to the McPhail trap; the included lures were: torula yeast, ammonium acetate, borax, putrescine, an antifreeze [based in propylene glycol], commercial grape juice, liquefied raisins, and an artificial grape-flavor drink. The combinations were compared against the traditional McPhail trap+torula yeast. During the 2004 winter, clear multilure traps with torula yeast attracted >4 times irradiated Mexican fruit flies than McPhail traps ($P=0.02$); however, these differences were no significant during the 2005 spring. In comparison with ammonium acetate+putrescine+antifreeze, liquefied raisins, the artificial grape-flavor drink, and torula yeast in the McPhail trap, the treatment with commercial grape juice had the highest capture of Mexican fruit flies ($P=0.001$). Our results have implications for the monitoring of the pest in Mexico.

Attraction and Conservation of Predacious Arthropods in Citrus Orchards of Northeast Mexico

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Presently, the citrus industry of northeast Mexico is at the risk of invasion by the brown citrus aphid, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), a pest that invaded the south of Mexico during February 2000. In order to favor the abundance and diversity of indigenous natural enemies that could attack invasive populations of the pest, we evaluated food sprays in young citrus trees, and weed management practices in a mature citrus orchard. Food sprays included brewer's yeast (*Saccharomyces cerevisiae*)+sugar, baker's dry yeast+sugar, powder milk+sugar. Weed management included the following treatments: a) control (no weeds), b) weeds allowed only under the canopy of the trees, and c) inter-row presence of weeds. In comparison with the control (trees sprayed with 1 l of water), trees that received the treatment based on powder milk (5 g)+ sugar(5 g) in 1 l of water/tree showed in the leaves significant high number of chrysopid eggs and larvae (mainly *Chrysoperla rufilabris* (Burmeister) and *Ceraeochrysa* sp. nr. *cincta* (Schneider) [Neuroptera: Chrysopidae]) (P=0.0009). Plots with weeds, either under the tree or in the inter-row area had a significant presence of spiders (Araneae: Araneidae, Salticidae, Tetragnathidae, Thomisidae) as well as beneficial insects (Chrysopidae and Coccinellidae) (P=0.01). Spiders were notably abundant in the trees (>6 times the number of beneficial insects). Citrus yield was not reduced by plant competition. In northeast Mexico, weed management in citrus orchards should consider that the abundance and diversity of beneficial arthropods associated with the presence of wild plants could contribute to the control of *T. citricida*.

Natural control of citrus blackfly in the main citrus producing states of Mexico

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Citrus blackfly, *Aleurocanthus woglumi* Ashby, is species originated in tropical and subtropical Asia, it was introduced into many countries of the world and became serious citrus pest. The introduction of non-indigenous parasitoids has led biological control of this pest. In México *A. woglumi* is widely distributed pest of *Citrus* spp. In 2004-2005, citrus blackfly had high populations in the states of Nuevo Leon, Tamaulipas and Veracruz: 3,304 hectares were infested. Three of four parasitic species introduced to Mexico against *A. woglumi* were established successfully: *Encarsia perplexa* Huang & Polaszek, *Encarsia colima* Myartseva (fam. Aphelinidae) and *Amitus hesperidum* Silvestri (fam. Platygasteridae). The most important role in biocontrol of *A. woglumi* is done by *Encarsia perplexa*. *Amitus hesperidum* is rather rare and *Encarsia colima* was found only in the state of Veracruz. In this period high parasitization in Tamaulipas and Nuevo Leon was observed. Complex of parasitoids reduced population densities of Citrus blackfly below economic damage levels.

Comparison of the expression of a cold responsive transcript in nine citrus species and *Poncirus trifoliata*

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One gene called citrus low temperature (*CLT*) responsive gene has been identified in *P. trifoliata*. It encodes a small highly hydrophobic protein believed to provide stability to the cell membrane during freezing temperatures. It has two transcripts; the *CLTa* and *CLTb*. *CLTb* is constitutively expressed while *CLTa* is expressed specifically when plants are subjected to low-temperatures and has an additional 98 nucleotides at the 3'-untranslated region (UTR). The objective of this study was to identify *CLTa*'s presence and transcription efficiency in other more susceptible citrus species with varying degrees of sensitivity to cold temperatures. Genomic analysis and RT-PCR were conducted in 9 citrus species and *P. trifoliata* with various degrees of cold tolerance to verify the presence and expression of this gene. All species studied contained the *CLT* gene in its genome and expressed equally *CLTb*. However, the expression of *CLTa* was time dependent, with the cold hardy species responding quicker than the cold sensitive ones.

Enhancing Water Use Efficiency of Grapefruit Production in LRGV

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On-farm management strategies to conserve water in the Lower Rio Grande Basin is of concern due to reduced water flows for irrigation during periods of drought and increased demand by rapid population growth in South Texas. Grapefruit (*Citrus paradisi Macfad.*) production is economically important for growers in South Texas, which has been traditionally under flood irrigation. The objective of this study was to analyze crop production and irrigation use efficiency (IUE) after converting mature grapefruit trees previously adapted to flood irrigation to drip and microjet spray irrigation practices. This study was performed at the Texas A&M University-Kingsville Citrus Center's South Farm located in Weslaco, TX. Grapefruit production was monitored from 2002 to 2004, where the impacts of flood, drip and microjet irrigation were assessed on grapefruit yield and irrigation use efficiency (IUE). It was found that by the second year of production after adaptation to low-water use systems, the grapefruit trees out produced flood irrigated trees, with a significantly greater IUE over flood irrigation. This demonstrates that as irrigation water becomes scarce and high cost to growers in South Texas, shifting to low water use systems like drip and microjet spray may be more logical and feasible over the long-term.

Searching Response of *Cheiracanthium inclusum* to Potential Cues Associated with Lepidopteran Eggs

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Cheiracanthium inclusum (Hentz) (Araneae: Miturgidae), a wandering spider, has been frequently observed feeding on the eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), an important cotton pest. Spiders are known to feed mainly on mobile prey, thus mechanisms used by wandering spiders to locate sessile prey remain understudied. Foraging techniques vary among species, especially between webbuilders and non-webbuilders. Vibratory and visual are among the main cues used by spiders to identify prey, however, other sensory modalities such as chemoreception are poorly understood. The objective of this study is to determine the types of cues utilized by *C. inclusum* to locate and identify *H. zea* eggs as prey. Preliminary results show that *C. inclusum* positively responds to substrates previously exposed to *H. zea* eggs and responds similarly to substrates containing moth scales. These results suggest that *C. inclusum* may be using a combination of contact and chemical cues from eggs and scales to recognize *H. zea* eggs as prey. In later tests, *C. inclusum* was observed to initiate searching behavior when exposed to substrates containing solvent washes of eggs and scales correspondingly. These results support the hypothesis that *C. inclusum* may in fact be using chemical cues, among others, to identify lepidopteran eggs as prey. Further studies identifying key chemical compounds may provide a better understanding on how *C. inclusum* perceives chemical cues and initiates searching behavior.

Ethylene Degreening and Citrus Fruit Decay

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Harvested, mature green citrus fruit are subjected to 2 to 5 ppm ethylene treatment for color development. Facilities are designed for this degreening treatment with many stacked bins of fruit at 82-85 °F and 90-95% relative humidity. One cycle/hr fresh air exchange is recommended to avoid carbon dioxide build-up. Citrus fruit are normally embedded with dormant fungal spores from the field; however, the ethylene treatment conditions would induce spore germination resulting fruit with stem-end rot disease. Degreening rooms with improper design and air quality parameters result in serious fruit rot and human health issues. Ethylene oxide reacts with carbon dioxide to produce carbon monoxide. Results showed most degreening facilities in south Texas need to be properly regulated for fruit rot management.

Population dynamics of the citrus blackfly parasitoids in Mexico

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Citrus blackfly (CBF) *Aleurocanthus woglumi* Ashby, is a serious citrus pest of South Asian origin it was detected in Mexico in 1935 and controlled by 1953. It damages citrus by sucking nutrients from foliage, which weakens the plants. CBF excrete honeydew on which sooty molds develop; sooty molds can severely impair leaf respiration and photosynthesis. The combined effects on the tree of blackfly feeding and the associated sooty mold can result un serious fruit yield reductions. Blackfly infestations are encountered today in the state of Tamaulipas, Nuevo Leon, San Luis Potosi and Veracruz, the main citrus producers in Mexico. The main objective of this study was to know the distribution and CBF infestation level and parasitization in the indicated states of Mexico. CBF presence has been confirmed in 2004-2005 in 3304 hectares, leaf samples from these groves showed infestation from 65 to 90 % and dissection of CBF immature indicated parasitization from the introduced parasitoid *Encarsia perlexa* ranged from 90 to 100 %.

Asymmetric Hybridization in Citrus

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Asymmetric hybridization in citrus allows overcome some of the problematic biological reproductive characteristics of Citrus such as apomixis, polyembryony, pollen or ovule sterility and sexual incompatibility. Gamma irradiation causes fragmentation and subsequent elimination of chromosomes in the donor genome allowing transferring of small portions of the donor genome to the receptor. Different doses of gamma irradiation were applied to protoplasts of different citrus cultivars and fused with non-irradiated protoplast of second citrus species using polyethylene glycol method. Doses from 30 to 250 gamma-rays were applied and different fusion combinations were performed. Iodoacetamine (IOA), an inhibitor of the mitotic-spindle assembly, was used in some of the receptor protoplasts to induce genome inactivation. Non-fused IOA treated cells cannot divide and usually degenerate. This way the hybrid cells had an advantage in development compared with the irradiated or IOA treated control protoplasts. Plantlets have been regenerated and confirmation of hybridization is underway through chromosome counting, random amplified polymorphic DNA analysis and genomic in situ hybridization.

Molecular Studies of *Phytophthora* in Citrus Orchards in the Valley

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Phytophthora infection causing foot rot and gummosis is common in the Lower Rio Grande Valley (LRGV) citrus orchards. No previous studies were conducted to characterize this fungus using molecular methods. We used ribosomal DNA (rDNA) analysis as a tool to characterize *Phytophthora* from root and soil samples. First round polymerase chain reaction (PCR) with Ph2 and ITS4 primers resulted in 700 base pair (bp) fragment, which is common for 14 *Phytophthora* species. Primers Pn5B-Pn6 and Pc2B-Pc7 are highly specific for *P. nicotianae* and *P. citrophthora* respectively. A highly sensitive nested PCR produced 120bp fragment with primers Pn5B-Pn6 and no amplification with primers Pc2B-Pc7. Similarity search at GenBank showed 100% identity to *P. nicotianae*. This concludes that *P. nicotianae* is the most prevalent species in LRGV citrus and it confirms previous non-molecular assessment.