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Mycorrhizal Inoculation of Tomato and Onion Transplants Improves Earliness

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Field experiments were conducted near Weslaco, Texas (Lat. 26° 8' N) between 1999 and 2001 in order to evaluate the field performance of pre- and post-mycorrhizal-inoculated tomato and onion transplants. In 1999 'Heatmaster' tomato (*Lycopersicon esculentum*, Mill.) transplants were inoculated at 17 kg/ha with *Glomes interadices* (Reforestation Technologies, Salinas, CA) at transplanting; in 2000 'Heatmaster' and 'Florida 47' plants were treated at transplanting and exposed to two irrigation regimes; and in 2001 'Heatmaster' plants were either pre- or post-transplant inoculated and grown in a light (Hebbronville) or heavy (Raymondville) textured soil. In all years, cumulative fruit yield from mycorrhizal-treated plants were significantly greater by the second and/or third harvests, but final season yield, fruit number and average fruit weight were usually similar to controls. Marketable yield and fruit number tended to improve when plants were treated with mycorrhizae. Onion (*Allium cepa*, L.) cultivars Granex 1015Y and Terlingua inoculated with mycorrhizae in a seedling mix or at transplanting and planted in Hebbronville and Raymondville soil series tended to have greater yields and accelerated maturation, but bulb soluble solids at harvest were similar when compared to uninoculated plants. Bulbs from mycorrhizal-treated plants were more uniform in diameter. Bulbs stored at 13.5° C for 120 days suffered less soluble solids and weight loss if they were from mycorrhizal-treated plants. Bulb sprouting was not affected by any treatment.

Interaction of Soil Texture, Light Intensity, and Cultivar on Leaf Nutrients in Mustard Greens

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A greenhouse experiment was conducted near Weslaco, Texas (Lat. 26° 8' N) between 17 Dec. 2001 and 14 Feb 2002 in order to evaluate the effect of soil type, light environment, and cultivar on mustard greens leaf nutrients. Cultivars Tendergreen and Florida Broadleaf (*Brassica juncea*) were sown in four soils, Carrizo Springs, Hebbronville, Hidalgo, and Raymondville (listed in increasing clay content), and grown in two environments. The first consisted of ambient light conditions of 770 kmol[•]m⁻² cumulative average hourly PAR and 19.0° C mean season temperature and the second consisted of 402 kmol PAR and 18.2° C, respectively. Mustard greens grown under 50% reduced PAR were lower in leaf transpiration and ascorbic acid, but higher in leaf area, chlorophyll, carotenoids, total N, NO₃, and most leaf mineral nutrients. Root fresh weight, but not top fresh weight was reduced by low light. Plants grown in heavier textured soil had increased leaf area and, when grown in reduced light, pigment and leaf nutrient levels were even higher in the higher clay soils. Ascorbate was highest in plants grown in the Carrizo Springs soil exposed to ambient light. Folate was not affected by cultivar, light, or soil type (P<0.05).

Some New Information on Citrus psorosis virus in Texas.

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Four major tree-killing freezes (in 1951, 1962, 1983 and 1989) drastically reduced the commercial citrus acreage in the Lower Rio Grande Valley. Indirectly, these freezes reduced the incidence of citrus trees with symptoms of citrus psorosis disease. The development and commercial success of a new (psorosis-free) grapefruit cultivar, 'Rio Red' through mutation breeding of seedlings derived budwood also helped to reduce the overall incidence of psorosis in new orchards in Texas. However, in the past 6 years, we have observed field symptoms of psorosis in new plantations (post-1989), old plantations (trees pruned after the 1983 freeze), and dooryard trees (post-1983). In a recent survey involving 2,513 grapefruit trees of various varieties, obvious psorosis incidence was 3.5%, with another 4.9% showing psorosis-like symptoms. Biological indexing of several trees was done by grafting young shoots onto sweet orange (Pineapple, Dweet tangor, Madame Vinous) and Duncan grapefruit indicator plants. Typical symptoms were observed on indicator plants 4-6 weeks after grafting. The pattern of adjacent infected trees along flood irrigated rows in a commercial citrus orchard indicates a possible natural spread of this disease. A related virus in lettuce was recently showed to be spread by *Olpidium*. Preliminary studies conducted involving examination and sectioning of feeder roots from symptomatic citrus trees and culturing of soil with baits showed the presence of *Olpidium*-like fungus with resting spores. The *Olpidium*-like fungus in feeder roots of symptomatic trees strengthens the hypothesis of natural transmission of *Citrus psorosis virus*. However, verification of the virus-vector relationship needs to be established.

Sclerenchyma Cell Deterioration of Citrus tristeza virus-infected Mexican Lime

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Mexican lime leaf veins infected with a severe *Citrus tristeza virus* (CTV) strain (CTV-3) and a mild strain (T-TX 8) were compared. It is a universal indicator plant for diagnosing virtually all strains of CTV which can cause tree death, stem pitting, or mild reactions. Many translucent areas in the vein are prominent in the leaves infected with a severe strain and less prominent with mild strains of CTV. Cross sections of veins from the prominently translucent, slightly translucent, non-translucent, and 'healthy' areas were compared for sclerenchyma cell degradation. These cells form a sheath around the leaf vascular bundles. They are thick-walled, lignified, and mineralized cells that provide mechanical support. Specimens for microscopy were prepared with free hand sections and stained with a Fungi-Fluor[™] and examined under a Nikon Eclipse TE 300 fluorescence microscope with a CoolSNAP- Procf monochrome camera. In the severe strain infected plant, sclerenchyma cell degradation was 63% of the total cells in sections from the prominent vein clearing area and 34-48% in less prominent areas. In the mild strain infected plant, the highest cell degradation was only 40%. Sections from non-translucent and >healthy=areas had all cells intact. This appears to be the first quantitative report of sclerenchyma cell degradation associated with CTV infection.

Effective New Lure Maximizes Anastrepha Fruit Fly Captures in North and South America

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A synthetic lure developed jointly by IPM Tech (Portland, Oregon) and USDA-ARS (Weslaco, Texas) surpassed expectations for both attractiveness to *Anastrepha* and longevity in the field. IPM Tech lures outperformed Biolure (Suterra, Inc.) 2- component lures advertised effective for Mexican fruit flies (*A. ludens*) 5 fold when used on sticky yellow panel traps in grapefruit orchards in south Texas. In Peru, IPM Tech lures tested on sticky bottle traps outclassed standard (SENASA) ammonium phosphate McPhail traps 20 to 1 for captures of South American fruit flies (*A. fraterculus*) and over 100 to nothing for Mediterranean fruit flies (*Ceratitis capitata*). IPM Tech lures lasted upwards of 16 weeks in these tests. The IPM Tech *Anastrepha* lure shows great promise as a tool for detection and monitoring of the Mexican fruit fly, other species of *Anastepha* and the Mediterranean fruit fly.

Monitoring Population Dynamics of *Thrips tabaci* (Thysanoptera: Thripidae) and predacious Natural Enemies on Onions in South Texas.

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The population dynamics and stage composition of *Thrips tabaci*, and predacious natural enemies in lambda-cyhalothrin and methomyl treated and untreated onion plants were determined during the spring season using the whole-plant sampling method. In addition, sticky blue and white plastic cup traps and CC traps were used for trapping thrips. Result from this study indicated that the whole-plants sampling with absolute numbers of *T. tabaci* on onion plants was the most accurate method, but it was also most time consuming. Sampling thrips adults could provide a relatively good estimation of the total thrips population on onion plants with 70-75% precision. Of the developmental stages of *T. tabaci* on onion plants, 76-85% were nymphs, <0.1% were pupae, and 15-24% were adults. Although thrips densities were significantly reduced by applications of the insecticides compared with those on untreated plants, thrips densities were still far exceed the economic threshold. Several species of predators were found on onion plants. *Orius insidiosus* (Say) was the most dominant species of predators, with 41.0 and 74.5% of total predators collected from insecticide-treated and untreated onion plants, respectively. However, it appears that they were not the major factor regulating thrips population. Numbers of predators were significantly reduced on insecticide-treated plants compared with those on untreated plants. Of the traps, the blue plastic cup traps caught the most thrips (19-23 thrips per trap per day), followed by the white cup traps (10-12 thrips per trap per day), and the CC traps only caught a few (<1 thrips per trap per day).

Quantifying and Predicting Onion Price Movements

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This paper presents estimates of the inverse-demand relationship between the price and quantity demanded for onions supplied from South Texas. The study is based on data from 1990-2001. Weekly onion price movements are negatively related to quantities shipped from South Texas as well as from competing regions. The regression shipment coefficients quantify the price impact per truckload shipped from specified regions, while the associated price flexibility estimates quantify price impacts in percentage terms. Weekly onion prices trend down from week to week within the average season, and are positively correlated with the previous week's price.

Detection of Phloxine B in Mexican Fruit Fly, Honey Bee and Honey

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A spectrophotometric method for detection of phloxine B (D&C Red 28), a phototoxic dye proposed as a replacement for malathion, in extracted tissues of the Mexican fruit fly, *Anastrepha ludens* Loew, (Diptera: Tephritidae), the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) and in honey was developed. Dye detection was increased with increasing pH from 6 to 13.7 in insect tissues or from 3.7 to 8 in honey with 2% sodium hydroxide. An LC₅₀ of 29.62 ppm phloxine B in 30% sucrose was obtained by feeding honey bees. A predictive model for dye in insect tissues and honey was developed. This study provides a forensic approach to determine if bees were killed or honey was contaminated from field sprays targeted to kill flies.

A Novel, Rapid, Sensitive and Continuous Spectrophotometric Assay for the Determination of Diamondback Moth General Esterase Activity

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Conventional methods to determine general esterase activity from insects are composed of a three-step process where the esterase is allowed to hydrolyze a 1-naphthyl acetate substrate, that reaction is quenched by a SDS detergent, and then a Fast Blue B dye complex is formed with 1-naphthol, the product of 1-naphthyl acetate hydrolysis. These methods measure dye-product complex rather than the product. Measurement of the dye-product complex can lead to inaccuracies in the determination of 1-naphthol concentration. A new method continuously monitors the formation of 1-naphthol with the hydrolysis of a general esterase substrate at 320 nm. The esterase activity was determined by the slope changes of absorbance over minutes. The 1-naphthol product from the esterase reaction was confirmed by HPLC. The optimum pH value of esterase ranged from 6.0 to 7.5 in a sodium phosphate buffer, suggesting that esterase be a weakly pH-dependent enzyme. Divalent Mg²⁺ enhanced esterase activity but monovalent K⁺ and Na⁺ inhibited it. The optimum temperature for esterase activity was from 33° to 42° C. Denaturation of the esterase protein occurred at 5° C or at 60° C. The K_m and V_{max} values of the esterase were 28±2 M and 6.0±0.1 M/min, respectively, at 37° C for 1-naphthyl acetate. The K_i value was 9±2 M using azadirachtin, an insecticide from neem tree, *Azadirachta indica* (A.Juss). Azadirachtin was a reversible competitive inhibitor of the esterase activity. The method provides a rapid and sensitive assay for general esterases extracted from a single diamondback moth in 1 to 10 minutes.

Molecular Detection of Transgenic Food Products

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One third of the maize (corn) cultured in 1998 in North America was genetically modified and according to estimates, within 10 years this proportion will increase to 75%. This has created a series of concerns within the agricultural food industry to know if their raw materials originate from Genetically Modified Organisms (GMO). One of the most effective and accepted technologies for the detection and characterization of introduced DNA sequences in transgenic plants has been the Polymerase Chain Reaction (PCR). Several DNA sequences can be detected and these can be classified into three categories: 1) sequences that regulate the expression of the gene (promotor 35S and terminator Tnos); 2) genetic markers, usually genes, that confer resistance to some antibiotics; 3) transgenes, widely used ones such as the Cry series genes from *Bacillus thuringiensis*. In our laboratory we have established the analysis of possible GMOs for diverse foods products, such as grains, flour, Atortillas@ and even processed food. The analyses include the detection of the zeine gene as a positive control of the reaction of the PCR in maize samples, the lectin gene of soya, and the *Cauliflower mosaic virus* 35S promoter, since it is one of the most widely promoters in the generation of GMOS. One of the key elements for success is genomic DNA extraction. The experience of different analyses of diverse maize products will be shown. So far, we are adapting the methods for Real-time PCR, which will be more accurate for mixture of samples.

Molecular Differentiation of Mild and Severe Citrus tristeza virus Strains

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Citrus tristeza virus (CTV) is the most important viral disease of citrus causing losses of more than 100 million trees worldwide. The virus is transmitted by *Toxoptera citricida* and infected grafts. Most visible symptoms caused by the CTV are decline and death of trees grafted on *Citrus aurantium* L. or *Citrus macrophylla*. Early detection of CTV has relied mainly on serology but increasingly on nucleic acids methods. Recent achievements include sequencing of the virus genome and transgenic citrus resistant to the virus. Our objective was to develop a nucleic acid method for differentiating CTV strains. In order to

accomplish our objective we amplified the virus coat protein gene (p25), and then we screened isolates for restriction enzyme polymorphisms. We found that *Hae* III distinguishes the mild from severe races. Furthermore, *Kpn* I seems to differentiate those strains causing stem pitting from those inducing decline. The multiple alignment of the analyzed amino acids sequences results in a dendogram separating the mild strains from severe ones. The sequence analysis of the gene p25 showed two possible amino acids, glycine and threonine in positions 49 and 63, respectively, that are conserved in the severe strains and thus may be involved in the pathogenicity of the CTV. The polymorphism of the 5 UTR was also analyzed using specific primers for mild and severe CTV races. Currently, we are in the process of collecting and analyzing higher number of p25 gene sequences and gathering data on its biological behavior, to further validate our results.

Masking the Effect of Inoculation of Tropical *Azospirillum brasilense* by Indigenous Azospirilla from a Semi-arid Zone

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Two maize (corn) field experiments with inoculant were carried out in the semi-arid region of Northeast Mexico, during the winter-spring and fall-autumn of 2001 growing seasons. The inoculant consisted of a mixture of *Azospirillum brasilense* strains isolated from maize roots in the tropical region of Mexico. The antibiotic resistance of the strain confirmed the establishment of the inoculated strains. The Azospirilla concentration was lower than that reported in rich soils, predominantly acidic, from temperate zones. But in all treatments, numbers of *Azospirillum* were similar with a maximum number of bacteria per gram of tissue of only 1 X 10³. All treatments gave a grain yield between 5 to 5.4 ton/ha, but did not significantly increase grain yield over that of the non- inoculated control plot. Failure to increased yield with *Azospirillum* could be due to contrasted environmental conditions in the region in compare to the bacteria's region of origin. Significant inoculation effects on grain yields were observed for the second experiment (Fall-Winter), in which a 27% of yield increase from 3.7 to 4.11 ton/ha was observed. The results of the present study over the two field experiments strongly indicate that a more detailed, study of plant genotype-*Azospirillum* spp and indigenous strain interaction are required. In addition, a better interaction with the plant is needed to allow better inoculation results of cereal crops in the arid or semi-arid region where environmental conditions strongly differ from the natural habitat of *A. brasilense*.

Seasonal Fluctuation of Aphid Predators Associated with Citrus in Northeast Mexico

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The citrus industry of northeast Mexico is at the risk of invasion by the brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae), the most efficient vector of *Citrus tristeza virus*, a pathogen that has caused the death of millions of citrus trees worldwide. In order to contribute in the establishment of a program for the biological control of *T. citricida* in northeast Mexico, the present study was carried out to determine the presence and abundance of aphid predators in the citrus of the region. After two years of samplings in citrus trees, the collected aphid predators were the green lacewings *Ceraeochrysa* sp. nr. *cincta* (Schneider), *Ceraeochrys valida* (Banks), *Chrysopa nigricornis* Burmeister, *Chrysopa quadripunctata* Burmeister, *Chrysoperla comanche* (Banks), *Chrysoperla externa* (Hagen), *Chrysoperla rufilabris* (Burmeister), *Eremochrysa* sp., and *Leucochrysa mexicana* (Banks) (Neuroptera: Chrysopidae), a species of brown lacewings (Neuroptera: Hemerobiidae), and the lady beetles *Cycloneda sanguinea* (L.), *Hippodamia convergens* Guer., and *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae). The chrysopids were significantly more abundant than the other predators. They were present mostly during the cool season of the year (from September to March) in the region. *C. rufilabris* was notably abundant during such period; however, the species was absent during the remaining of the year. In contrast, *C. valida* was collected under more variable climatic conditions. Unlike chrysopids, presence of coccinellids was not associated with weather conditions. The study has implications for conservation of the different aphid predators and for taking advantage of their presence in the citrus orchards.

Flash Chromatographic Separation and Isolation of Structurally Similar Flavanoid Glucosides

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Flavanoid glucosides have been shown to prevent certain chronic diseases such as cancer, diseases of peripheral circulation and to lower blood pressure. Citrus molasses, a waste residue, has a large concentration of limonoid glucosides and flavanoid glucosides. Isolation of the pure glucosides from the mixture proves to be a complex problem due to their structural similarity. A rapid separation technique using a combination of adsorption chromatography and flash chromatography was designed to isolate these compounds from the mixture. The citrus molasses was de-pectinised and passed through ion-exchange resins to obtain an extract rich in glucosides. The enriched extract was agitated under cold conditions to separate the glucosides, which are present in maximum concentration. The freeze-dried extract was further separated using flash chromatographic technique. A small four-step gradient method using acetonitrile and water as mobile phase was developed. Structurally very similar flavanoid glucosides can be separated to the extent of 94%. This system can be used as a rapid technique for separation of closely related flavanoid glucosides.

Development of a *Trichoderma* Based Biofungicide Suitable for Northern Tamaulipas Weather Conditions

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The northeast region of México comprises the states of Tamaulipas, Nuevo Leon and Coahuila, having common border with the south of Texas. Typical weather along this region varies from dry to hot sub-tropical, and specifically in the state of Tamaulipas we found mild alkaline and saline soils. Crops cultivated in this part of the country are mainly sorghum, cotton, maize, bean and okra. Disease-causing fungi commonly found are *Aspergillus, Fusarium, Claviceps* and *Macrophomina*. Some of these fungi can be predated by several species of the genera *Trichoderma*. Although several *Trichoderma* biocontrol products are reported, and some are even available in the market, the microorganisms used on them were isolated from regions of milder weather. We hence have focused on the isolation, characterization and formulation a biocontrol agent product suitable for the crops (mainly sorghum) of the north of Tamaulipas. We are using the fungus *Macrophomina phaseolina* as a disease causing agent model (Charcoal Stalk Rot), isolated from sorghum and maize at Río Bravo cropping zone, as well as isolates from other parts of México and Italy. During the first stage of this project, we isolated 9 *Trichoderma* strains, which had been characterized morphologically and by means of AFLP's (amplified fragment length polymorphisms). We had also performed confrontation experiments for *in vitro* determination of the efficacy of biocontrol of the isolates at increasing temperatures, measuring the degree of growth inhibition as well as assessing the degree on invasion by means of an image analyzer. These results have allowed us to choose the most effective *Trichoderma* isolates to be used in the next stage *in planta* in the greenhouse.

Agave durangensis Adaptation on Four Substrates

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The micro-culture of plant tissues is an alternative technique for Agave plant production. The advantage of plants produced by this system is the feasibility to program the plant production, obtaining healthy plants, industrial scope and selected plants for physiological genetic or commercial properties. In our laboratory we are making *Agave durangensis* cultures in a synthetic medium. After this, plants are transferred to plastic trays (with 98 holes) using 4 different substrates. The plant survival rates observed were 67, 92, 77 and 70% corresponding to clay, peat moss, clay-peat mixed (50-50%) and natural soil respectively. With respect to plant size, only 40% of plants less than 1 cm in height survive, compared plants 1 cm or more in height, which have a survival rate of about 98%.

Use of a Hydroponic System for Lettuce Production in Reynosa, Tamaulipas

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The hydroponic system (NFT: nutrient film technique) was evaluated for the production of French lettuce (*Lactuca sativa* L. var. *longifolia*) under greenhouse conditions in the cycle autumn-winter at Reynosa, Tamps. The seed plot was developed in autumn and the production in winter. The French lettuce production was 0.55 kg/plant (23.6 ton/ha/cycle) and 0.40 kg/plant (23.6 ton/ha/cycle) when the frame of plantation was 4.3 and 5.9 plant/m², respectively. The Roman lettuce production was 0.55 kg/plant (32.4 ton/ha/cycle) with a frame of plantation was 5.9 plant/m². The water consumption was 13.8 L/plant, corresponding 93.0% of this consumption to the evapo-transpiration and the rest to vegetative material. The cycle time was 27 days in the seed plot, 59 days in the hydroponic system until the harvest was finished.

Agave potatorum Micropropagation

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Tamaulipas has a high demand for agave plants for its fast growing tequila and mezcal industry. At present, the supply comes mainly from rootstocks, but micro-propagated plants are gaining more relevance each day. Our Center has been micro-propagating *Agave durangensis* for the last two years. Based on this experience, we are starting to micro-propagate *A. potatorum* cultured plants in Murachige and Skoog medium modified in our center. Our source of plant tissue is stem and meristematic areas of wild plants previously quarantined in our laboratories. Incubation is at 25° C under 1000 lux of brightness. The results show that 68% of vegetative tissues are generating healthy plants. On the other hand, 16% of plant tissues are dying and 16% contaminated. We now have fully developed plants ready to be transferred and evaluated in the field. Hopefully, they will become the source of high quality material to supply the tequila and mezcal industry of our state.