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RGVHS07-001

Water Conservation Initiative Project for the Lower Rio Grande Valley of Texas

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Abstract: Water conservation for on-farm use incorporating state-of-the-art technologies are helping farmers in drought potential areas. Technologies including using alternative methods to deliver irrigation water to crops using surge valve with polypipe delivery, drip irrigation and micro-jet spray irrigation practices. Collecting information such as: crop (varieties), acres irrigated, irrigation method- drip tape (emitter spacing, rate), micro-jet (configuration of jets/tree and rate) and soil types and classifications will enable future speculation on best fit uses of irrigation technology for farmers. By monitoring yields of crops in relation to different types of irrigation will indicate to farmers of the Lower Rio Grande Valley (LRGV) that sustainable fruit and vegetable production is possible with water saving techniques.

RGVHS07-002

Molecular Detection and Prevalence of Citrus Viroids in <st1:State w:st="on">Texas

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Abstract. Viroids are graft- or mechanically transmissible agents, disseminated through budding. Biological indexing of commercially important citrus cultivars grown in the Lower Rio Grande Valley of Texas showed that many are infected with citrus viroids. Most of these trees carried more than one viroid. In most cases, the infected trees are asymptomatic carriers because sour orange, the predominant rootstock used in Texas , does not show symptoms of viroid infection. Detection of viroids through biological indexing on sensitive indicator plants followed by sequential polyacrylamide gel electrophoresis (sPAGE) is the gold standard but is time consuming and requires plants to be kept at optimum conditions. A conditional use of RT-PCR provides an efficient and alternative detection method of citrus viroids for use in the Texas virus-free citrus budwood certification program. RT-PCR could be useful in <st1:place w:st="on"> Texas to help expedite the detection of viroids before conducting the final biological indexing. Using RT-PCR, we could detect, clone, and sequence full-length viroids of *Citrus exocortis viroid* (CEVd), *Hop stunt viroid* (HSVd) (both cachexia and non-cachexia variants), *Citrus viroid-III* (citrus dwarfing viroid), and *Citrus viroid-IV* (citrus bark cracking viroid) from a collection of viroid-inoculated grapefruit plants. Based on our results, RT-PCR can be a conditional substitute for biological indexing of virus-free mother trees in foundation block and shoot-tip grafted trees in the virus-free budwood program. A positive RT-PCR result has a serendipitous value because those trees can be discarded from the pool prior to expensive biological indexing.

RGVHS07-003

Title: **Evaluating inoculation techniques for gummy stem blight disease of cantaloupe.**

Authors: **R. Saldana (1), A. Garza (2), A. Davelos Baines (2), M. Miller (1), and C.R. Little (2)**

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Abstract:

Didymella bryoniae causes gummy stem blight of melons including cantaloupe (*Cucumis melo reticulata*). The pathogen is soilborne and usually exists in association with plant debris at or near the soil surface. The principle objective of this investigation was to test and compare six cantaloupe seedling inoculation techniques using *D. bryoniae*. All of the inoculation techniques utilized conidia as inoculum and included: (1) foliar spray of seedlings, (2) foliar spray with wounding of seedling cotyledon leaves, (3) soaking cantaloupe seed in inoculum prior to planting, (4) mixing inoculum into soil prior to planting, (5) infesting soil around seed at planting, and (6) incorporation of infected plant debris into soil prior to planting. Foliar spray with and without wounding resulted in the highest levels of stem lesions and dead plants 14 days after the experiment was initiated. Inoculation of soil at planting resulted in the highest disease severity levels for soil-based inoculation techniques. The most effective technique will be used to facilitate growth chamber and greenhouse studies to test the efficacy of soilborne biocontrol agents to control gummy stem blight.

RGVHS07-004

Standardizing Paraquat as a Selection Tool for Drought Tolerance in Sugarcane

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Abstract

Increasing scarcity of irrigation water is one compelling reason for breeding drought resistance in sugarcane. But drought resistant varieties can also be used in well-irrigated areas, for the purpose of productivity and water conservation. Selection of such varieties demands an efficient and quick selection strategy. The correlation between paraquat (PQ) resistance and drought tolerance makes PQ a suitable selection tool in plant improvement for drought tolerance. However, before using PQ as a screening tool, appropriate standardization and procedures must be established for a given species. The objective of the current study was to determine the threshold concentration and exposure duration of PQ needed to distinguish between drought tolerant and susceptible genotypes of sugarcane. Leaf disks (1.3-cm diameter) were floated in PQ solutions (0 μM, 50 μM, 100 μM, 150 μM, 200 μM) for different durations (0 h, 24 h, 48 h, 72 h and 96 h) and the whole experiment was conducted under the constant light exposure of 120 $\mu mol.m^{-2}.s^{-1}$. Chlorophyll degradation (a measure of PQ resistance) was monitored using a rapid, nondestructive approach (SPAD-502 Minolta Chlorophyll meter), and a spectrophotometer. During the initial three days after exposure to PQ, SPAD and spectrophotometer estimates of leaf chlorophyll content were not correlated (0.077) however, after day 4, the estimates were strongly correlated (0.713). The SPAD meter was not sensitive to minor changes in chlorophyll that occurred in the initial three days. The 150 μM PQ solution was found to be the most optimum screening concentration since it triggered quantifiable changes in chlorophyll content within 24 h as measured with the spectrophotometer. Higher concentration (200 μM) did not give any better results than 150 μM PQ concentration at the given light level. Using this same concentration, the SPAD meter could detect changes in chlorophyll concentration only after 72 h.

RGVHS07-005

Title: Identification of False Spider Mite (Brevipalpus) species and detection of Citrus Leprosis Virus in the Lower Rio Grand Valley

Authors: Jesus Mata Jr., M. Setamou, J.V. French, J. da Graça, and E.S. Louzada

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Abstract:

In the Lower Rio Grand Valley of Texas (LRGV), three known species of false spider mite (FSM): *Brevipalpus phoenicis, B. californicus* and*B. obovatus* are potential vectors of the Citrus Leprosis Virus (CLV). Identification of *Brevipalpus*to species level is difficult because of their similar morphology and small size. Genetic fingerprinting methods such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) are accurate and rapid for the identification of *Brevipalpus* spp. Leaves and fruit damaged by FSM feeding will be collected and analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) in an attempt to detect CLV in the LRGV.

RGVHS07-006

Towards a Broad–Spectrum Disease Resistance in Citrus

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With over 26,000 acres grown in the Lower Rio Grande Valley and revenue of more than $93 million, citrus represents a big part of the <st1:place w:st="on"> Texas economy. However, diseases like Huanglongbing (HLB) and Citrus canker are already present in <st1:State w:st="on">Florida and it could easily reach <st1:State w:st="on">Texas soon. The Citrus tristeza virus (CTV) is also a threat to Texas citrus industry because the most used rootstock/scion combination is very susceptible to the virus. The most efficient vector for CTV, the brown citrus aphid, is already present in Florida and parts of Mexico . Management solutions have been used, but they do not provide a permanent solution to the problem. The gene defense-no-death1 (dnd1) of Arabidopsis thaliana was reported to be involved in the first signals of the hypersensitive response (HR). Silencing of the dnd1 gene in A. thaliana inhibits cell death but induces broad-spectrum disease resistance. Recently, we isolated a dnd1-like gene in citrus. This citrus dnd1-like (c-dnd1) gene is a member of a family of genes that encodes for cyclic nucleotide gated ion channel (CNGC) proteins. Our research will be directed to isolate and characterize the remaining members of the c-dnd1 gene family and to over-express or silence them in sour orange (Citrus aurantium) in an attempt to induce broad-spectrum disease resistance.

RGVHS07-007

Title: Host-plant selection of Asian Citrus Psyllid (*Diaphorina Citri*)

Authors: Dr. Mamoudou Setamou, Adrian Sanchez

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Abstract:

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera; Psyllidae) is a phytophagous insect that vectors *Candidatus Liberibacter*, the etiological agent of citrus greening disease. Citrus greening is one of the most economically important citrus diseases in the world that can destroy a citrus industry within a short period of time. Although *D. citri* exhibits differential host preferences for plants in the Rutaceae family including Citrus spp, the mechanism of its host selection are poorly understood. The present study is undertaken to gather data to shed light on plant cues used by *D. citri* in its host selection process. Several experiments will be performed, including behavioral assays to test psyllid response to olfactory stimuli in an olfactometer, and visual stimuli emanating from colored sticky traps. Results of this study will improve the understanding of *D. citri*plant colonization and provide baseline information that can be used in the design of pest control measures.

RGVHS07-008

Title: **Analysis of gene expression during cold acclimation in *Citrus***

Authors: **Arturo Saldivar and Dr. Eliezer Louzada**

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Abstract

Citrus is one of the top commercially important crops in the world. Most of the citrus varieties are cold sensitive. *Citrus aurantiifolia* (Christm.) Swingle (Mexican Lime) is a very cold-sensitive citrus variety which cannot tolerate low temperatures. On the other hand, *Poncirus trifoliata* (L.) Raf. is a monotypic genus related to *Citrus* that can tolerate temperatures of -30ºC when fully acclimated. Consequently, *P. trifoliate*has been used to try to improve the cold tolerance of some citrus varieties. In the present study, 900 putative up-regulated genes from a subtraction library will be analyzed using Reverse Northern Dot-Blot. cDNA from acclimated and non-acclimated *P. trifoliata* (PT), Mexican lime (ML), and Mexican lime grafted on *P. trifoliata* (ML-P) is going to be use as probe. The gene sequences will be compared to those deposit at the GenBank database. By comparing the genes expressed in PT, ML, and ML-P we expect to be able to identify those that are more important during cold acclimation. At the same time we expect to figure out if signals from transcription factors in PT will be transferred to ML. The identification of these cold-regulated genes is important for the understanding of cold acclimation in *Citrus*. The application of this knowledge to *Citrus* will help improve the cold tolerance of plants that are more susceptible to freeze damage.

RGVHS07-009

Identification of AFLP Polymorphism in Grapefruit Cultivars Correlated to Flesh Color

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Abstract

Through natural and induced mutations of grapefruit (*Citrus paradisi*) seedlings and budwood, numerous cultivars have been produced from <st1:place w:st="on">Duncan grapefruit. In 1984 <st1:State w:st="on">Texas A&I University released Rio Red grapefruit from irradiated seedlings of Ruby Red. This red grapefruit contains phytochemicals such as lycopene, beta-carotene, limonoid glucosides, naringin and vitamin C. The red pigmentation of grapefruit is attributed to the presence of carotenoids, primarily lycopene. Research has shown that carotenoids help prevent cancers and enhance the immune system. The purpose of this study is to identify molecular markers correlated to flesh color in different grapefruit cultivars. Five grapefruit varieties, Duncan , White Marsh, Thompson, Ruby Red, and Texas Red* (Rio Red budsport) will be analyzed. Amplified Fragment Length Polymorphism (AFLP) will be performed on genomic DNA using an IRDye® flourescent AFLP® kit (Li-Cor®, <st1:City w:st="on">Lincoln , NE , <st1:country-region w:st="on">USA). Polymorphic molecular markers with a possible correlation to flesh color will be excised, sequenced, and compared to the NCBI GenBank database. The identification of molecular markers will help us correlate the lycopene biosynthesis pathway which may affect the flesh color of the fruit.

RGVHS07-010

Use of spinosad for control of *Anastrepha ludens*(Diptera: Tephritidae) in citrus orchards of northeast Mexico

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The insecticide malathion has been frequently used to control fruit flies; however, recently, its use has been commonly questioned. Nowadays, the insecticide spinosad is considered an alternative to the application of malathion for the control of such pests. The objective of this study was to evaluate the insecticide spinosad in sprays and in bait stations, against Mexican fruit fly, *Anastrepha ludens* Loew (Diptera: Tephritidae) in northeast Mexico. As an attractant for the insect, we incorporated a bait recently developed by the USDA-ARS. In a first trial, we evaluated the treatments 40, 20, and 10 ml/tree/biweekly of the mixture: 80 ppm of spinosad + the new attractant; as a regional control we sprayed 175 ml/tree/week of the mixture: <st1:metricconverter ProductID="1.0 l" w:st="on">1.0 l of malathion 50 CE, <st1:metricconverter ProductID="4 L" w:st="on">4 l of hydrolized protein and <st1:metricconverter ProductID="96 L" w:st="on">96 l of water. In a second trail, spinosad was evaluated in bait stations; the treatments were: 40 and 20 bait stations/ha, the above regional control, and an absolute check. There were no statistical differences among the sprays of spinosad and malathion in the average number of Mexican fruit flies captured; though, spinosad sprayed in 40 ml and 10 ml/tree registered significant lower fruit infestations by *A. ludens* (0.4 and 0%, respectively) than the regional control (2.4%). Treatments of spinosad in 20 or 40 bait stations/ha were more effective to reduce the number of flies than the sprays of malathion. Our results have important implications for the management of *A. ludens* in the region.

RGVHS07-011

Perspectives for biological control of *Toxoptera citricida* (Homoptera: Aphididae) in Mexico

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The brown citrus aphid, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae) invaded the south of <st1:place w:st="on"> Mexico during February 2000. This aphidis the most efficient vector of Citrus Tristeza Virus. In <st1:country-region w:st="on">Mexico , the initial invasion of the pest was confronted with massive releases of the exotic predator *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). This study was performed in order to offer alternatives to the biological control of *T. citricida* in <st1:place w:st="on"> Mexico , through the use of indigenous species of predators and entomopathogenic fungi. Our findings showed that the release of *Ceraeochrysa* sp. nr. *cincta*(Schneider), or *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) had control of *T. citricida* only at low densities. At high densities of the pest, the aphids were preyed by an assemblage of beneficial arthropods (Neuroptera: Chrysopidae & Hemerobiidae; Coleoptera: Coccinellidae; Diptera: Syrphidae) that interfered with the performance of the released species. Practices of conservation biological control (food sprays and weed management) had significant effects favoring the activity of diverse natural enemies of the pest. Native isolates of *Beauveria bassiana* (Bals.) Vuill., *Lecanicillium lecanii* (Zimm.) Gams & Zare, and*Paecilomyces fumosoroseus* (Wize) Brown & Smith produced high mortality of *T. citricida* in lab and field. Selected isolates of *B. bassiana, and P. fumosoroseus* did not cause significant differences in mortality of *T. citricida* predators in comparison with the control. In the field, *P. fumosoroseus* isolate AMBAS1 constantly caused high mortality of the pest. Our results offer new perspectives for the control of *T. citricida* in Mexico.

RGVHS07-012

Expression analysis of stress-responsive genes in sugarcane using a functional genomics approach

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Cross-talk between signaling pathways is an effective mechanism by which plants regulate stress-responsive genes. Using a functional genomics approach, we analyzed part of the sugarcane transcriptome corresponding to stress-responsive genes in stem tissue. A stem-expressed cDNA library consisting of 13,824 clones was constructed and screened to select 229 candidate genes. Using microarray analysis, changes in the transcription profiles of the 229 genes were monitored in response to: (a) infection with a compatible sorghum mosaic virus (SrMV) strain, or (b) treatment with the defense-signaling molecules, salicylic acid (SA), methyl jasmonate (MeJA) or jasmonic acid (JA). These treatments significantly altered the level of specific gene transcripts, many derived from known and putative defense-related genes. One expression profiling group consisted of 24 genes that were 2-fold co-induced by SA and JA. A majority of these genes are implicated in plant defense responses and encode dirigent (DIR) proteins. A second group comprised 45 genes that are 2-fold induced by SA and co-repressed by JA and MeJA. These encode more than 30 DIR proteins, one defensive *o*-methyltransferase and one anti-microbial chitinase. A third group consisted of 56 genes that are 2-fold induced by MeJA and co-repressed by SA and JA. These include cell maintenance and development genes, translationally controlled tumor proteins and 2 DIR proteins. Interestingly, many genes from the first two groups were 2-fold down-regulated upon SrMV infection. The microarray analysis was validated by quantitative PCR. Our transcriptional profiling data revealed a high level of coordinated defense responses in sugarcane, demonstrating the existence of a network of regulatory interaction among different stress signaling pathways.

RGVHS07-013

Effect of Compost Application in South Texas Grapefruit Production

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Citrus is grown in approximately 27,000 acres in the Lower Rio Grande Valley (LRGV), Texas with majority of it under flood irrigation. But due to rapid urban development and semi arid conditions there has been a decline in irrigation water supplies. Moreover, soil in LRGV has high clay content which causes water logging under flood irrigation and is unfavorable for efficient root growth. Because of limited supplies and concerns with the water logging conditions new strategies to increase the irrigation use efficiency are being sought to sustain citrus production. A field experiment was conducted from 2003 to 2005, located at the Texas A&M University-Kingsville, Citrus Center South Farm in Weslaco, Texas with 17 year old Rio Red grapefruit trees (*Citrus paradisi* Macfad) comparing compost and non compost treatment under drip and micro-jet spray irrigation systems. After one year of compost application, a trend of higher crop production was observed in composted trees compared to non-composted trees in both the irrigation systems in 2004 and 2005 harvest years. Similar trend was also noticed in root density correlating with improved soil nutrient and water uptake leading to improved yield over time. This suggests that annual compost application under low water use systems may be ideal for improving citrus yield in long term. This may be due to composts ability to improve the water holding capacity of soil. In all the harvest years non-fertilized control treatment trees produced significantly lower yields in both number and weight suggesting that fertilization is important to maintain yield values.

RGVHS07-014

Inhibition of Bacterial Cell-Cell Communication and Biofilm formation by Citrus Bioactive Compounds

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Certain limonoids found in citrus have been shown to have human health promoting properties. In the present study, five citrus limonoids were tested for their inhibitory activity on the autoinducer-1 and autoinducer-2 mediated communication pathways in *Vibrio harveyi*. The AI-1 and AI-2 bioluminescence assays were conducted using 96 well microtiter plate and *Vibriyo harveyi* strain BB170 and BB886 as reporter strains. Limonin and Isoobacunoic acid glucoside were found to inhibit the AI-2 mediated cell-cell communication by more than 50%, at the lowest concentration tested (6.25 µg/ml). Limonin and Isoobacunoic acid were antagonistic to the AI-1 mediated cell-cell communication. Bacterial cell-cell communication has been shown to regulate various processes like biofilm formation, bioluminescence, virulence factor, antibiotic production, sporulation and competence for DNA uptake. Among these, biofilm formation is one of the most important traits, which is responsible for recurrence of the bacterial infections. Therefore, antagonistic activity of limonoids towards biofilm formation of *E. coli* 0157:H7 was tested using 96 well microtiter plate bioassay. Ichangin was found to be most effective in preventing the biofilm formation in *E. coli* among the tested limonoids. This project is based upon work supported by the USDA CSREES IFAFS # 2001 52102 02294 and USDA-CSREES # 2005-34402-14401 "Designing Foods for Health" through the Vegetable & Fruit Improvement Center.

RGVHS07-015

Yeasts as Biocontrol Agents of Citrus Phytopathogenic Fungi

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Abstract

Some citrus epiphytic yeasts can be used as biocontrol agents due to their high growth rate, coupled most of the time to the production of degradative enzymes (mycoparasitism) and/or fungitoxic metabolites (antibiosis). Furthermore, they are attractive from the process point of view due to the fact that these microorganisms are usually very easily cultivated and formulated. The use of these unicellular fungi is specially suited for post-harvest storage. In this work we analyzed the biocontrol performance of 7 yeasts (named as LCBG-1 to 7) isolated from the epiphytic mycoflora of limes (*Citrus limon* var. Eureka) over 3 phytopathogenic fungi (*Penicillium digitatum, Mucor* sp. and *Trichoderma*sp) isolated from diseased limes. *In vitro* and *in vivo* tests demonstrated that all isolates were able to control *Mucor* sp at the three levels of inocula used: $1x10^6$, $1x10^7$and $1x10^8$ yeast mL^{-1}, causing at least a 20 % reduction on the fungus radial growth rate. For *P. digitatum* we observed that the best biocontrol was shown by isolate LCBG-30, which was identified as *Debaryomyces hansenii* based on its ITS1-5.8S-ITS2 sequence. Finally, *Trichoderma* sp was the most resistant fungus, and only the isolate TCBG-3 (identified as *Pichia guilliermondii* LCBG-3) was able to control it in at least a 50 %. We postulate that this yeast is able to produce a fungitoxic metabolite, besides being a good space competitor. Currently we are characterizing the metabolic and genetic profiles of *P. guilliermondii* LCBG-3 when it is induced by *P. digitatum* cell walls and *in vivo* confrontation.

RGVHS07-016

Structural Differences of Citrus Limonoids Influence Induction of Glutathione S-Transferase and Quinone Reductase

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Citrus limonoids have been shown to induce phase II enzyme activity. In the present study, three citrus limonoid, such as limonin, limonin glucoside, and deacetyl nomilinic acid glucoside and two synthetic conmounds, defuran limonin and limonin-7-methoxime, were tested for the ability to induce glutathione S-transferase (GST) and quinone reductase (QR) against chemical carcinogenic agents. Mice were treated with limonoids dissolved in a 1:1 ratio of DMSO and corn oil by oral gavage. After four treatments, mice were sacrificed and organs were harvested and homogenized in PBS. Organ homogenates were used for the assay of GST and QR activity. When compared to control, GST activity against CDNB was induced by 67% in deacetyl nomilinic acid glucoside in lung homogenates, by 55% and 32% in limonin-7-methoxime treated intestine and liver homogenates respectively. GST activity against 4NQO was induced by 270% and 51% in limonin-7-methoxime treated liver and stomach homogenate respectively, and the deacetyl nomilinic acid glucoside treatment group increase GST activity by 55%. QR activity was induced by limonin-7-methoxime by 72, 65 and 32% in intestine, liver and lung homogenates respectively, and by defuran limonin treatment group by 45% in lung homogenates. Our results indicated that modification to the functional groups of the limonoid structure have an influence in their ability on inducing phase II enzyme activity. These finding are indicative of a possible mechanism for the prevention of cancer by aiding in detoxification of xenobiotics. This project is based upon work supported by the USDA-CSREES IFAFS#2001-52102-02294 and USDA-CSREES#2005-34402-14401 "Designing Foods for Health" through the Vegetable & Fruit Improvement Center.

RGVHS07-017

Biochemical Properties of Furocoumarins from Grapefruits

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Red grapefruit is the state fruit of <st1:State w:st="on">Texas which plays major role in the <st1:PlaceName w:st="on">Rio Grande Valley agriculture economy. Grapefruit juice contains several health promoting compounds and carries America heart associations “heart check” food mark. However, grapefruit juice increases the bioavailability of certain drugs mainly by reducing the first pass metabolism of drugs. Unique group of compounds called furocoumarins are the bioactive principles responsible for the grapefruit-drug interaction. Bioactive furocoumarins such as dihydroxybergamottin, paradisin A, bergamottin, bergaptol and geranylcoumarin were isolated with a combination of chromatographic techniques and characterized with various analytical studies such as HPLC, NMR and LCMS. Effects of isolated furocoumarins were evaluated at different concentrations on CYP3A4, CYP2C9 and CYP2D6 isoenzymes activity. Among the five furocoumarins tested, the inhibitory potency was in the order of paradisin A > dihydroxybergamottin > bergamottin > bergaptol > geranylcoumarin at 0.1 µM to 0.1 mM concentrations. The IC_{50} value was lowest for paradisin A for CYP3A4 with 0.11 µM followed by DHB for CYP2C9 with 1.58 µM. The levels of these compounds were also monitored for variation due to season and growing location. Ray red showed the lowest levels of all three furocoumarins and <st1:City w:st="on">duncan contains the highest amount of DHB and bergamottin, where as the highest levels of paradisin A was observed in star ruby. Both DHB and bergamottin showed decrease in levels as season progresses, while paradisin A did not show variation in both varieties. This work is supported by the USDA-CSREES under Agreement USDA-IFAFS # 2001-52102-02294 and USDA # 2005-34402-14401 "Designing Foods for Health" through the Vegetable and <st1:PlaceName w:st="on">Fruit Improvement Center.

RGVHS07-018

Methods of genomic DNA isolation to facilitate sequencing of polydnavirus genomes

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Methods of polydnavirus (PDV) DNA isolation from calyx fluid's *Cotesia flavipes* (a parasitoid of several pyralid larvae such as the sugarcane borer, *Diatraea saccharalis*), and *C. congregata* (a parasitoid of several sphingid larvae such as the tobacco hornworm, *Manduca sexta*) wasps were investigated to facilitate ongoing genome sequencing projects. The long-term goal of PDV genome research is to identify novel molecules which may serve as biopesticides to target insect pests that threaten agriculture. One strategy we are currently developing is to bioengineer insect pathogenic viruses with PDV gene(s) from the parasitoid *C. congregata*, to enhance the baculovirus's virulence for agricultural insect pests. Genetic alteration of insect-borne baculoviruses (e.g. *Autographa californica* M nucleopolyhedrosis virus) with PDV sequences will likely broaden their host range and increase their speed-of-kill thereby increasing their effectiveness in killing targeted insect pests. The genes encoding peptides or proteins of PDV origin will facilitate development of biopesticides which induce developmental arrest of the lepidopteran host. This strategy will also reduce our dependence on chemical pesticides which have significant deleterious effects on the agricultural environment.

RGVHS07-019

Sorghum mosaic virus HC-Pro and sugarcane proteins affecting posttranscriptional gene silencing in monocots

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HC-Pro is a well known viral protein that suppresses RNA silencing in plants. We confirmed that *Sorghum mosaic virus* (SrMV) infection in a transgenic sugarcane line showing post-transcriptional gene silencing, induced the expression of the silenced transgene (GUS). Also, the introduction of SrMV P1/HC-Pro into another transgenic line which is post-transcriptionally silenced for SrMV coat protein (CP) resulted in the accumulation of SrMV CP RNA. These data confirmed that SrMV P1/HC-Pro acts as a suppressor of RNA silencing in sugarcane, one of the most economically important crops worldwide. In order to investigate cellular component(s) involved in RNA silencing and its suppression in sugarcane, HC-Pro was used as a bait in a yeast-two-hybrid assay to screen cDNA expression library constructed from a transgenic line of the sugarcane hybrid cultivar CP65-357 showing RNA silencing for the SrMV CP gene. Yeast-two-hybrid screening identified several cellular proteins as interactors with HC-Pro. One of them is a ca. 22KD protein that preferentially binds to RNA. *In vitro* binding assays such as pulldown and farwestern assays further confirmed that SrMV HC-Pro interacts with the 22KD protein. Yeast-two-hybrid screening of the same cDNA expression library with the 22KD protein identified a ca. 33KD protein as an interactor which shows high identity with 14-3-3 proteins. *In vivo* interactions between HC-Pro, the 22KD and the 33KD protein were investigated by fusion with various fluorescent proteins. The possible involvement of the sugarcane proteins in RNA silencing and its suppression is under investigation.