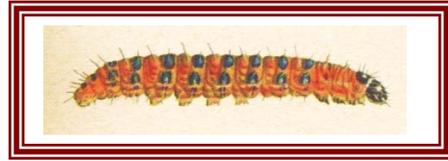
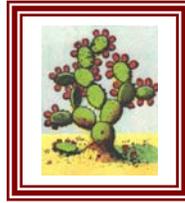


***Cactoblastis cactorum* Activities Report for Jan-Feb-Mar 2007**



For past reports and more information, see the PPQ Cactus Moth website at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/cactoblastis/index.shtml

PLEASE NOTE: THIS IS A NEW URL FOR OUR WEBSITE, Change your bookmarks !

Joel Floyd, USDA-APHIS-PPQ-EDP, Riverdale, MD

CONFERENCE CALL: A conference call was held with SAGARPA to discuss changing duties of officials in Mexico City with regard to the program, and the March visit to Isla Mujeres and Cancun by ARS researchers.

VISIT TO ISLA MUJERES: ARS scientists Stephen Hight and Jim Carpenter visited Cancun and Isla Mujeres, Mexico to review the ongoing eradication program on the island and discuss the recent trap catches in the Cancun area. No larval infestations have been detected on the mainland in Cancun or along the coast of Quintana Roo or Yucatan states. Discussions are underway to complete the up front work necessary to send sterile insects to the area in the future.

INTERNATIONAL CONFERENCE: The International Cactoblastis Conference will take place May 8-10th at the Desert Botanical Garden in Phoenix, Arizona. All arrangements with the hotel were finalized. The agenda and registration information can be found at

http://www.aphis.usda.gov/plant_health/plant_pest_info/cactoblastis/conference.shtml

FUNDING FROM MEXICO: The agreement to receive the 2007 funds for the Cactus moth cooperative program from SAGARPA through NAPPO was finalized and the funds received from NAPPO in March.

PPQ FIELD ACTIVITY: Field work resumed in January at Bon Secour NWR, AL, with physical removal of hosts. Maurice Duffel worked with Bill Bryant of PPQ AL and Connie Ramos of APHIS-CPHST in MS. Other APHIS-CPHST personnel have provided excellent assistance, including Craig Hinton who has worked in host removal at Dauphin Island and Little Dauphin Island, AL. We have had additional assistance, from Robert Smith, Joe Dawson, and Gene Bohannon, Marsh Lowe, Mary Collins and Sue Von Drasek.

After receiving environmental clearance from US Fish and Wildlife Service at Bon Secour and Ft. Morgan Historical Park, Maurice Duffel was instrumental in having a front-end loader operator place heavy screen mesh on the bottom of the rake fitting of the

loader, instead of the normal bucket arrangement. This arrangement allowed for a more efficient, less costly host removal process by allowing soil and sand attached to the removed cacti to sift through and return to the ground. Nearly all host material was removed from the Ft Morgan area in a few days. Remaining host material at Bon Secour is being removed by hand. This effort should significantly reduce the cactus moth population and the availability of oviposition sites. The summary of host material removed at Ft, Morgan can be seen below.

Opuntia Host Removal Totals from Ft. Morgan/Bon Secour NWR, AL

2006		2007
Hand Removal	Machine removal	Machine Removal
1.9 tons	42.05 tons	50.26 tons

SURVEY: Richard Brown identified moths from 104 traps placed in Arizona nurseries, Grand Bay Savannah NWR, and Puerto Rico. All were negative for cactus moth except those present in traps from Puerto Rico. Richard also provided *C. cactorum* genitalia preparations to SAGARPA to assist with identification of adult trap captures in Mexico.

REGULATION: The Technical Panel on *Opuntia* Nursery Stock visited 5 nurseries in Florida in late February to evaluate a possible nursery stock certification program for Florida nurseries wishing to ship host plants to non-infested states. The panel’s evaluation will provide information and scientific justification to write the requirements into the domestic regulation already under development.

OUTREACH: Newspaper articles about cactus moth appeared in various locations including one from the AP Mexico City about adult trap captures on the mainland. This story appeared in some Australian newspapers:

http://biz.yahoo.com/ap/070215/mexico_threatened_cactus.html?.v=1

Another AP story in Albuquerque, NM highlighting the cooperative program: <http://www.casperstartribune.net/articles/2007/04/08/news/regional/3094a3a98b73c56c872572b40064b4f2.txt>

A version taken from this story appeared in the Tucson Citizen with added erroneous information that the *C. cactorum* occurs in Mississippi, Louisiana, and Texas: <http://www.tucsoncitizen.com/ss/local/46705.php>

TECHNICAL LIAISON: Stephanie Bloem collected and compiled all reports for program activities for January-March and facilitated communication with SAGARPA on a number of occasions.

George Schneider, FDACS-DPI, Gainesville, FL

Accomplishments and activities: The Biological Control Rearing Facility (BCRF) received additional cladodes from Edinburg, TX and egg sticks from Tifton, GA in February. Rearing was started in 100 larval containers. Initial infestation of cladodes was

higher than in previous trials and the majority of containers had live larvae through March. A total of 1,544 pupae have been recovered thus far. The smaller size of these cladodes is likely responsible for the increased yield.

We completed modification of 400 larval development containers and the first set of adult moth colony eclosion cages was received in early February. The cement used to fasten the screening material was not suitable for the larger cages. After consultation with Dow Chemical we evaluated 2 other products and chose one of Dow's silicon based adhesives that will apply easily and withstand repeated washings.

The necessary warehouse space has been cleared to allow placement and easy access to this unit. The design of the adult moth scale collection unit is progressing well with a representative from Aztec Plastics and several of our staff members having traveled to Tifton, GA to study the system for incorporation of ideas into our unit.

The egg stick incubator scheduled to arrive in March has been delayed a few weeks, but should arrive by mid-April. Rearing on artificial diet will commence shortly thereafter. The Waring blender has been received. Quotes for the rest of the infrastructure for large scale mass rearing is being obtained and a budget for the program's next fiscal year is being developed.

Stephen Hight, USDA-ARS, Tallahassee, FL
Jim Carpenter, USDA-ARS, Tifton, GA

SIT VALIDATION. Traps were serviced 1-2 times per month in January and February at Pensacola Beach, Ft. Morgan, Dauphin Island, and Little Dauphin Island. Weekly servicing began at all sites by mid-March. No wild males were captured at Dauphin or Little Dauphin Islands January-March. Wild males were captured the last 2 weeks in March at both Pensacola Beach and Ft. Morgan. The first releases of irradiated *C. cactorum* were made the first week of April. Total and average trap captures of wild *C. cactorum* are presented in Table 1. The average number of wild moths per trap in March is based on 4 weekly averages. Weekly captures at Ft. Morgan are presented in Table 2. Sanitation was conducted at Dauphin and Little Dauphin Islands during January and February. Approximately four tons of material was hand-removed. Two moderately infested plants found on Little Dauphin Island were harvested, returned to the lab, and insects reared to evaluate F1 sterility presence.

Table 1. Wild *Cactoblastis cactorum* (Cc) caught in traps during March 2007.

Location	Dauphin Is., AL	Little Dauphin Is., AL	Ft. Morgan, AL	Pensacola Beach, FL
# Traps	53	5	16	69
# Wild Cc	0	0	6	31
Avg. # Wild Cc/Trap/Month	0	0	0.1	0.1

Table 2. Weekly male *Cactoblastis cactorum* (Cc) trap capture, # of male and female irradiated moths released, and percent irradiated males recaptured at Ft. Morgan, AL, January - March 2007.

MONTH	WEEK OF YEAR	Cc CAPTURED		irradiated Cc RELEASED		% irradiated ♂ Cc RECAPTURED
		WILD ♂ Cc	irradiated ♂ Cc	♂	♀	
January	2	0	--	--	--	--
February	9	0	--	--	--	--
March	11	0	--	--	--	--
March	12	1	--	--	--	--
March	13	5	--	--	--	--

ECOLOGICAL AND QUALITY CONTROL FIELD STUDIES. Flight Periods and Degree-Day Model. A single male was collected the first week of January at the south Florida site. Males were not caught again until the first week of February, but were caught every week since then through March. No males were collected from any of the other sites during January or February (north Florida, coastal Georgia, and coastal South Carolina), but captures were made each week beginning mid-March.

Flight Studies (collaboration with ETH Zurich). An experiment was initiated to evaluate the flight potential of different generations of *C. cactorum*. Pads containing mature larvae were collected in February and March at naturally infested site in Florida. Pads were placed in screened boxes in an outside location. Pupae were removed weekly, de-silked, and sorted by gender. Pupae were shipped to Switzerland for flight evaluation.

VISIT TO MEXICO. Stephen Hight and Jim Carpenter traveled to Mexico in early March to review the progress of cactus moth eradication efforts in Isla Mujeres. Two days were spent on Isla Mujeres evaluating progress in host plant removal, trap service, sentinel plant placement and status, and searching for and removing newly infested host plants. An informational meeting was held to review the status of eradication and trapping efforts, review preliminary results of a door-to-door survey of all properties on Isla Mujeres. Three days were spent in the Cancun area. One day was spent at the office of the Comité Estatal de Sanidad Vegetal de Quintana Roo transferring trapping technology, training personnel in identification, and reviewing trap mapping and data collection methodology and efforts. A second day was used to review trap placement in the Cancun area, from Isla Blanca to the southern end of the Cancun hotel zone. We also reviewed placement of 40 additional traps and efforts to find infested plants around traps that captured males on the mainland. A third day was spent on Isla Contoy surveying plants and setting up traps. A final wrap-up meeting was held on Saturday morning before we returned to USA.

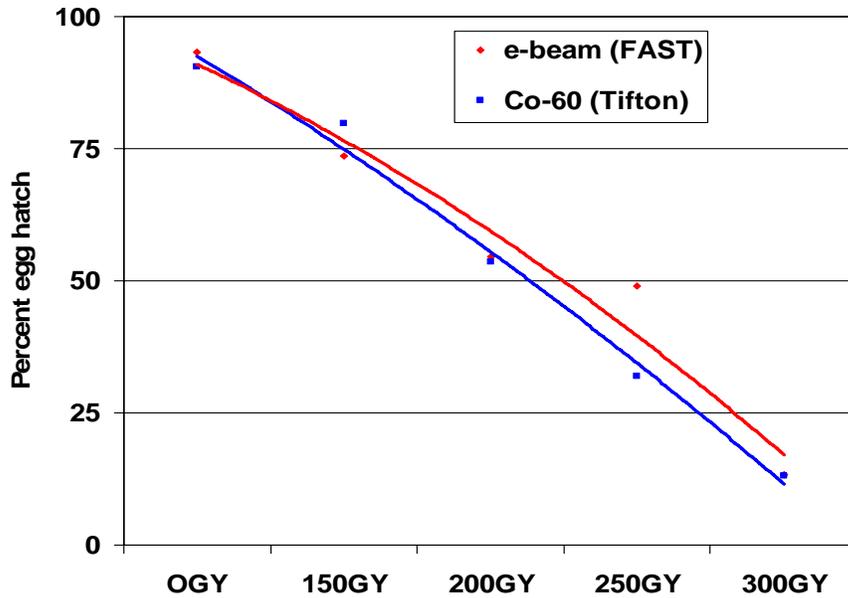
CACTOBLASTIS REARING. Drafts of the Standard Operating Procedures (SOPs) for rearing cactus moths on cladodes and on artificial diet have been written and given to Florida DPI. Although the SOPs will continue to be modified as more research data and input from DPI is obtained, current PDF versions are available.

Cladode rearing. Rearing was increased to the maximum level of production within the space available. Because production on cladode is more reliable, we chose to rely on this rearing method to rear moths for irradiation and release during the Spring flight. Production figures for February and March are as follows ...

	Eggs collected	Larvae on cladodes	Pupae collected	Adults emerged
Feb.	128,623	309,261	6,895	5,000
Mar.	75,921	128,623	23,860	17,895

Artificial diet rearing and diet trials. Trials to evaluate the effects of cooked vs. uncooked diets, the effects of reduced amounts of methyl paraben, and the effects of diet pH (4.0-5) on cactus moth production were conducted. Diet trials also evaluated diets supplemented with sterols (cholesterol and sitosterol) and minerals in low pH diet (4.0-4.7). We also assayed the incorporation of potassium sorbate and Beck's Salt to provide essential vitamins and minerals to the diet. Lastly, we evaluated incorporation of cactus and carrot flour into low pH diet. Preliminary findings, not yet supported by statistical analysis, are summarized as follows: (1) mean female pupal weights and percent survival were not increased when larvae were reared on diets with lower pH and no methyl paraben, (2) adding Beck's salt, substituting potassium sorbate for sorbic acid and adding more cholesterol to the standard diet, did not increase female pupal weights or % survival, however, adding sitosterol did appear to be beneficial, (3) mean female pupal weights and % survival were increased when larvae developed on diets containing fresh cactus cladodes, freeze-dried cactus cladode powder, or freeze-dried carrot powder, and (4) results varied greatly between trials, and between treatment replications largely as a result of sporadic disease.

RADIATION BIOLOGY TRIALS. In cooperation with Carl Gillis, FL Accelerator Services and Technology, DPI, Gainesville, FL, we evaluated the accelerator to sterilize cactus moths. The target dose (200 Gy) was calibrated to national standards. Radiation biology results verify that the dose delivered by the accelerator provides the appropriate biological response as compared to Co-60.



ADDITIONAL ACTIVITIES. Trapping Beyond Leading Edge. Traps were sent to collaborators who continue monitoring for *C. cactorum*. These include: Mississippi (Grand Bay National Estuarine Research Reserve and Gulf Shores National Seashore); Texas (APHIS-PPQ, in Galveston and Corpus Christi); and Arizona (AZ Department of Agriculture).

S. Dorn, M. Sarvary, ETH, Zurich, Switzerland

Dispersal Studies. The report “Laboratory studies of *Cactoblastis cactorum* flight capacity” was finalized and sent to the funding agencies. Results provide new insights into diel activity and comparative flight performance of male and female cactus moths.

We held a conference call with the participation of Drs. S. Bloem, K Bloem, S. Hight, J. Carpenter, where results of flight assessment of virgin and mated moths were discussed and research plans for the April – August 2007 were finalized. The main objective of this research is to test the hypothesis that different environmental conditions experienced by pre-pupal stages in winter and summer influence adult flight performance differently. Moths from the first and the second field generations will be tested in the flight mill with laboratory-reared individuals tested in parallel with each field generation as a control. The first draft of our first manuscript was completed and sent to Drs. Bloem and Carpenter for comments.

R. Heath, N. Epsky, USDA-ARS-SHRS Laboratory, Miami, Florida

Accomplishments and activities. Two shipments of pupae were received from Tifton. Total numbers were: Jan 5 - ~500 females, ~500 males; Jan 12 - 494 females, 504 males.

We received the new GC-MS and developed a method for large volume injection that resulted in increased sensitivity of detection. Samples are analyzed using the Agilent 6890N GC and a 5975 MSD quadrupole mass spectrometer system (GC/MS). The instrument is equipped with a programmable temperature venting (PTV) large volume injector capable of cryogenically focusing a large volume (50ul) of a diluted sample into the head of the column to detect trace amounts of material. The instrument parameters were set as follows: The PTV injector was cooled down to 0°C for an initial time of 4.2 min using liquid N₂. Injector temperature was increased to 320°C at a rate of 200°C/min with a holding time of 2 min. A vent flow of helium was established at a rate of 400ml/min for 4 min, and a purge flow of 400ml/min was added from 4-6 min. An HP-5ms 30m x 0.25mm x 0.25um (Agilent) was used for analysis with helium as the carrier gas at a constant flow of 1.2 ml/min. The GC oven was cryogenically cooled to 0°C for 1 min using liquid N₂. The temperature was then ramped to 250°C at 20°C/min and held for 10.5 min. The transfer line to the MSD was kept at 250°C throughout the analysis.

Samples were analyzed using electron impact (EI) mass spectrometry with a multiplier voltage of 1494. Temperatures for the MS quadrupole and source were set at 150°C and 230°C, respectively. The MSD was tuned using spectral tune parameters to be compatible with the NIST library for comparison. The MSD acquisition mode was set for both scan and single ion monitoring (SIM) in the range of 50-300 m/z. The targeted ions for SIM were 192m/z and 194 m/z. An initial delay of 8 min was set for data acquisition in order to prevent solvent damage to the MSD filament. Putative cactus moth synthetic attractants were used as standards to determine the retention times and fragmentation patterns of our compounds of interest.

When analyzing putative cactus moth pheromones of known concentration, the individual components were identified at a very low concentrations (200 pg in 50 ul = 4 pg/ul, Fig. 1). The quadrupole GC/MS system provides high sensitivity and a very low detection limit. It also offers high matching capabilities with the NIST library. In a gland extract sample from Nov. 22, 2006, for example, some of the putative pheromones are easily seen from the Total Ion Count chromatogram (Fig. 2). Chromatograms of diagnostic ions 192 and 194 were helpful, yet not necessary, to confirm their presence.

Four sets of gland extracts were obtained from female moths in January, which consisted of 28, 23, and 44 glands. In analyzing these gland extracts we found a large reduction in pheromone (Fig. 3) in comparison with those obtained from females in November 2006 (Fig. 2). The peaks of interest are so small in the January 2007 extracts that their presence could only be confirmed through diagnostic ion chromatograms. Thus, we have determined that there may be seasonal differences in amount of pheromone produced that may compromise the ability to analyze either gland extracts or volatile chemical collections during the winter months. We have stopped receiving shipments of moths from Tifton to both allow ARS-Tifton to build up sufficient numbers of moths for shipment to Miami as well as to delay additional chemical collections until the spring, a time that coincides with natural cactus moth population increase.

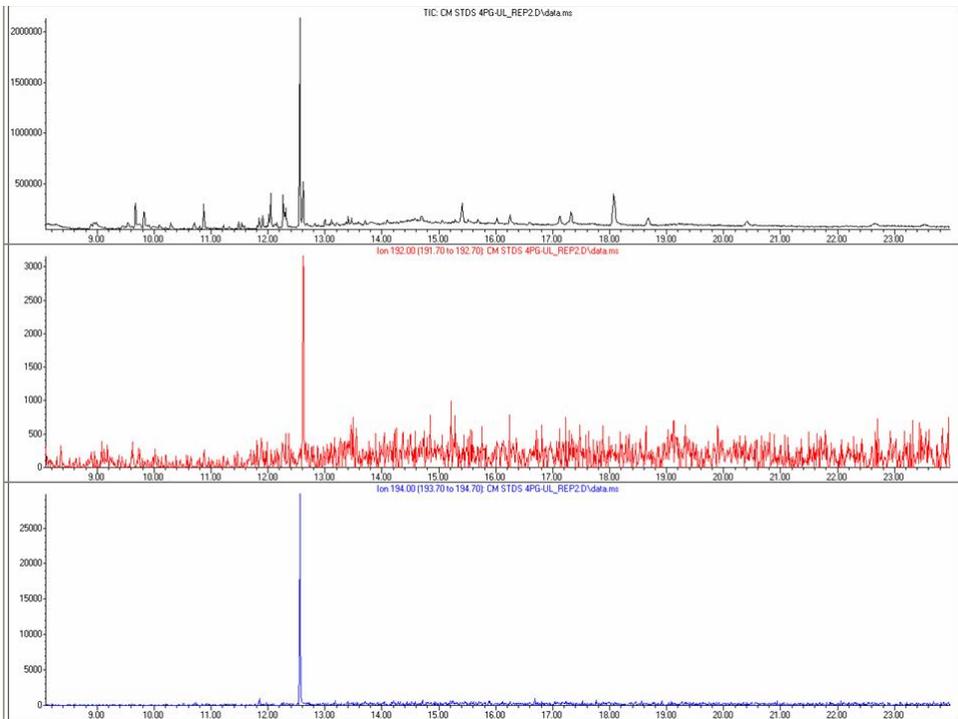


Figure 1. GC-MS of cactus moth standard (top), and two diagnostic ions (middle – 192, bottom – 194)

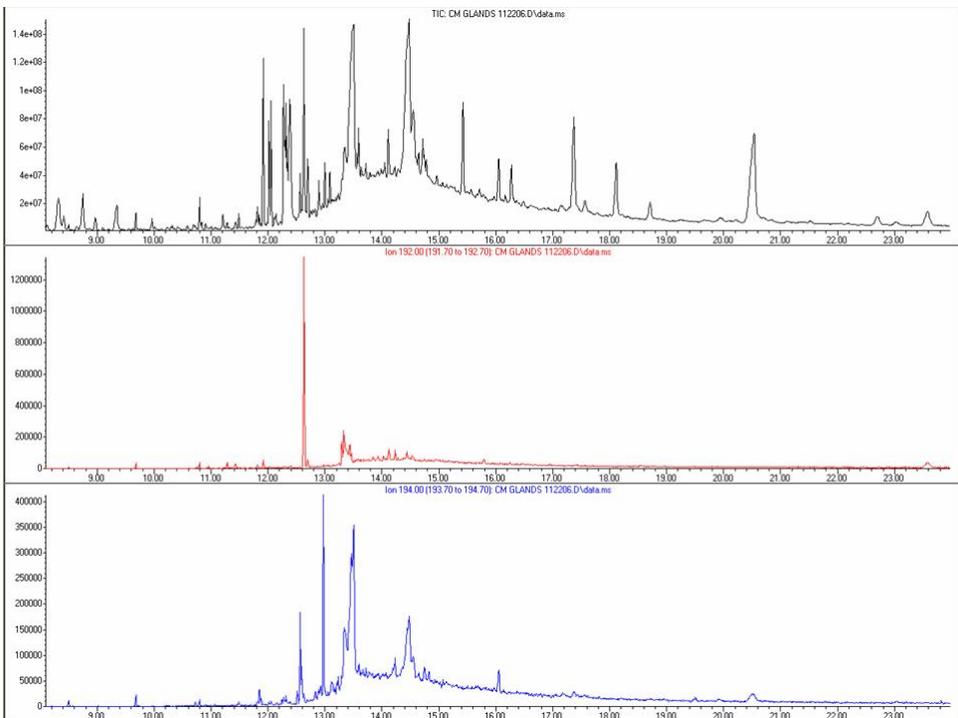


Figure 2. GC-MS of Nov. 22, 2006 gland extract from cactus moth females (top), and two diagnostic ions (middle – 192, bottom – 194)

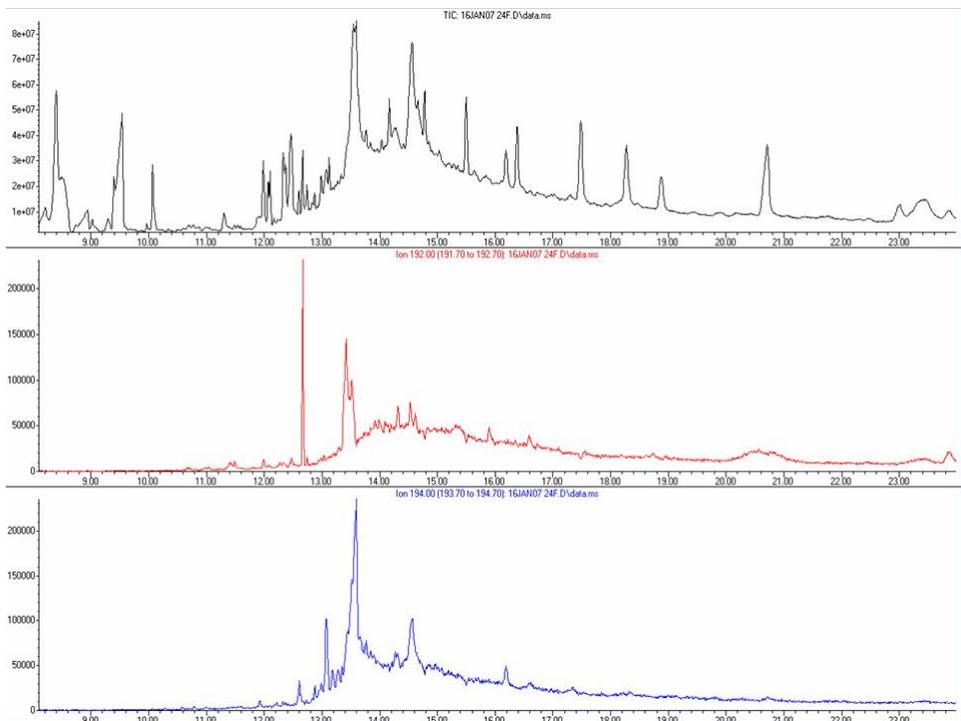


Figure 3. GC-MS of Jan. 16, 2007 gland extract from cactus moth females (top), and two diagnostic ions (middle – 192, bottom – 194)