20(1):50;Fonseca, *et.al.*,1994.Fitopatol. Bras.19(supl.):290). PLRV is one of the most disseminated *Luteoviridae* among wild and cultivated solanaceous species in the states of São Paulo and Paraná (Souza-Dias et al., 1993 Summa Phytopat.19(2):80-85). Attempts to evaluate a possible immuno relationship between CVMV and PLRV was carried out by DAS-ELISA using polyclonal IgG with homologous conjugate (EMBRAPA-CNPH kit). The results for 6 isolates of CVMV: 2 from the State of São Paulo and 4 from Paraná were all negatives. While neither visualization nor isolation and purification of CVMV have been accomplished, it seems to be worth to determine a possible antiserum relationship among the *Luteovidae* family species and take advantages on serological analyses for further studies on transmission, host range and varietal assessment of this not well characterized virus.

PL 8 - *COWPEA APHID-BORNE MOSAIC VIRUS* (CABMV) IS WIDESPREAD IN PASSIONFRUIT IN BRAZIL AND CAUSES PASSIONFRUIT WOODINESS.

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Passionfruit woodiness is one of the most economically important diseases of passionfruit, in all areas where it occurs. *Passionfruit woodiness virus* (PWV), a member of the family *Potyviridae*, genus *Potyvirus*, has been described as the causal agent of the disease in several countries, including Brazil. In South Africa, a potyvirus previously described as PWV was actually identified as a strain of *Cowpea aphid-borne mosaic virus* (CABMV) after phylogenetic analysis of the capsid protein gene and 3' non-translated region (3'NTR). Brazilian isolates of PWV have been previously characterized in terms of their biological and serological properties. However, molecular data had not yet been obtained. We have sequenced and analyzed the CP and 3'NTR of a collection of Brazilian isolates of PWV from the main passionfruit-producing states, including Minas Gerais, São Paulo, Bahia, Pernambuco, Paraíba and Pará. The results show that all isolates are members of the same species, CABMV. The Brazilian passionfruit isolates of CABMV are closely related to the South African isolate, and also to Brazilian isolates of CABMV infecting peanuts and cowpea. Interestingly, all CABMV isolates from passionfruit analyzed are capable of infecting passionfruit and cowpea. However, CABMV isolates from peanut and cowpea do not infect passionfruit. Together, these results indicate that CABMV is widespread in passionfruit in Brazil, causing passionfruit woodiness. The biological properties of these viruses indicate that they comprise a distinct strain, apart from CABMV isolates from peanut and cowpea. It remains to be determined whether PWV actually occurs in passionfruit in Brazil. Molecular analysis of a larger number of isolates will be necessary to answer this question.

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PL 9 - *CUCUMBER MOSAIC VIRUS* IN *EUCHARIS GRANDIFLORA* (*AMARYLLIDACEAE*).

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Eucharis grandiflora is a tropical plant native to the Colombian Andes, whose hybrids are cultivated for cut flowers and pot foliage. Although it has been cultivated for a long time in Brazil, there is no information on virus disease in this species. However, *E. grandiflora* has been found showing virus-like symptoms, characterized by foliar mosaic. Affected samples were used for electron microscopic observations, biological, serological and molecular studies. Elongated, Potyviridae-like and isometric (ca 30 nm in diameter) particles were observed in negatively stained foliar preparations. Since some inoculated plants reacted with mosaic, line-pattern, blistering and foliar distortion, DAS-ELISA was performed with antisera against Tobacco streak virus and *Cucumber mosaic virus* (CMV) (sub-groups I and II), showing positive reaction to CMV-I only. It is worth mentioning that only the isometric virus was transmitted to host plants. RT-PCR was performed using CMV specific primers and one fragment with about 800 bp was obtained. This result corroborates the fact that CMV is widely spread in ornamentals, mainly in those with vegetative propagation.

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PL 10 - DETERMINATION OF VIRUS/VECTOR INTERACTION OF A NEW BEGOMOVIRUS ISOLATED FROM TOMATO IN THE STATE OF GOIAS, BRAZIL.

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A begomovirus named GO-ANPL was obtained from tomato plants collected in orchards in Anápolis, State of Goiás. The plants showed mosaic symptoms, intervein clearing, leaf curling and growth reduction. To study virus/vector (*Bemisia argentifolii*) interaction, the acquisition access period (AAP), the inoculation access period (IAP), and the latent period were

determined by transferring five whiteflies per plant using tomato cv. Santa Clara as a host. For the AAP and IAP, nine different time periods were tested: 15 min, 30 min, 1, 2, 4, 8, 16, 20 and 24 h. The vector was able to transmit the virus after 15 min AAP, resulting in 6% of infected plants. The rate of transmission increased as the length of AAP, reaching 65% within 24 h. Regarding the IAP, it was observed 18% of infected plants after 30 min. The infection rate increased to 67% after an IAP of 24 h. The latent period was considered to be 16 h, when 3% of the inoculated plants were infected. To detect the GO-ANPL isolate in the vector, more than 2.500 specimens were tested by PCR. The presence of the virus was detected in the vector from the 1st to the 4th instar grown on infected plants, in adults under different AAPs and in adults from immature stages that were reared on infected tomato plants. No virus was found in eggs from aviruliferous females that had been laid on infected plants. The GO-ANPL isolate was transmitted to the progeny of viruliferous females, since the virus was detected in all stages of insect development from eggs to adults. However, no virus transmission was observed from these adults. High frequency of viral detection was observed in newly emerged adults from immature forms reared on virus-infected plants. These adults infected 33% of tomato plants in virus transmission assays performed subsequently. The results of the virus retention, the AAP, IAP, latent period and vector virus detection, indicates that the interaction between virus and vector starts at early stages of insect development. The higher levels of GO-ANPL isolate transmission efficiency with the longer AAP or IAP fit the persistent circulative mode of virus transmission.

PL 11 - FUNCTIONAL DISSECTION OF THE TMV (*TOBACCO MOSAIC VIRUS*) REPLICASE USING GREEN FLUORESCENT PROTEIN.

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Transgenic plants containing segments of the TMV replicase displayed resistance against several different tobamoviruses in a non-homology dependent fashion. There are two different forms of protection derived from segments of the TMV replicase genes. A low level derived from replicase RNA sequences via a sequence-specific host defense mechanism, termed post-transcriptional gene silencing (PTGS), and a high level derived from translatable segments of the 54-Kda polymerase domain. It seems that multiple mechanisms may contribute to the resistance conferred by TMV replicase domains. The goal of this work is to investigate the functions of specific replicase domains and how these functions can be utilized to confer protection in transgenic plants. Initially a conserved nuclear localization signal (NLS), encoded by the amino terminus of the 126 kDa protein, was investigated for its functionality using fusion constructs containing the green fluorescent protein (GFP). Fusion of the amino terminal 63 amino acids of the 126 kDa protein, containing the NLS, to a ß-glucuronidase-GFP open reading frame (ORF) directed the accumulation of fluorescence to the nucleus. It did not happen with the constructs lacking the NLS or containing a mutated NLS. Deletion constructs with the NLS motif were created and fused to the N-terminus of the GFP ORF. Two GFP fusion constructs (126^{1-178}-GFP, mw = 71.9-kDa) containing the first 178 and 388 N-terminal amino acids of the 126 kDa protein, respectively, were found to primarily localize to the nucleus but could also be observed associated as strands within the cytoplasm. In contrast, fusion constructs carrying the first 781 amino acids or the full-length 126 kDa ORF (126^{1-781}-GFP, mw = 115,7 kDa and FL126-GFP, mW152,9 kDa) did not localized to the nucleus but instead associated with the endoplasmic reticulum (ER), forming spot-like inclusions. It indicates that regions of the 126-kDa protein beyond amino acid 388 act in a dominate fashion over the N-terminal NLS to prevent the nuclear localization of the 126-kDa protein. Another 126-kDa-GFP fusion construct containing a non-functional NLS mutation also localized to ER but did not form inclusions. In addition, TMV mutant containing the same non-functional 126-kDa NLS failed to replicate in protoplasts. These findings suggest that the NLS contributes to the cellular localization of the 126-kDa protein and may play an important role in virus replication.

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PL 12 - IDENTIFICATION OF *BANANA STREAK VIRUS* STRAINS THROUGH ANALYSIS OF PCR AMPLICONS.

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BSV belongs to the family *Caulimoviridae* (genus *Badnavirus*) and banana is its natural host. The banana crop has considerable economic importance in Brazil, but no BSV diagnoses molecular method has been utilized to index banana germplasm in this country. A polymerase chain reaction (PCR) assay using degenerate primers, designed to amplify Badnavirus genus, has been standardized to detect and characterize BSV strains in banana cultivars from Brazil. The cycling parameters consisted of an initial denaturation cycle of 94 °C for 5 min., 42 °C for 2 min., 72 °C for 3 min.; followed by 25 cycles of 94 °C for 1 min., 42 °C for 2 min., 72 °C for 3 min., and finally, 1 extension cycle of 94 °C for 1 min., 42 °C for 2 min., 72 °C for 10 min. A screening of DNA samples isolated from banana leaf crude sap of plants collected in Brazil was carried out. The virus was detected in diploid (AA - 'Khai nai on'), triploids (AAA - 'Caipira', 'Grand Naine', 'Nanicão'; AAB - 'Maçã', 'Mysore', 'Prata', 'Prata anã', 'Terrinha' e 'Thap maeo'; ABB -