Hemipteran-Plant Interactions Symposium

Proceedings of the 2011

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Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ)

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Section 1

Phloem physiology and phloem-feeding insects

SMALL RNA RESPONSES TO APHID FEEDING IN RESISTANT AND SUSCEPTIBLE INTERACTIONS

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Plants respond to insect herbivory with a complex array of induced physiological changes including defense mechanisms that result in decreased herbivore fitness. A single dominant resistance gene (*Vat*) determines resistance to *Aphis gossypii* (cotton-melon aphid) and *A. gossypii* transmission of non-persistent mosaic viruses in *Cucumis melo* (melon). Resistance reduces aphid fitness; shortening aphid lifespan and resulting in smaller aphids that produce significantly fewer progeny. Genome expression analyses have identified specific patterns of gene expression in nearly isogenic aphid resistant and susceptible melon plants in response to *A. gossypii* feeding. Experiments were designed to determine whether small RNAs regulate gene expression under the biotic stress caused by aphid feeding and are components of the resistance mechanism both as gene expression regulators and as plant defense molecules. To this end small RNA libraries were constructed and analyzed and small regulatory RNAs were identified in both melon and *A. gossypii*. Quantatative real-time PCR experiments identified microRNAs that were simply aphid responsive, and a smaller number that showed a differential response in the resistant interaction. Analysis of the *A. gossypii* library data showed a number of plant phloem microRNAs were transferred to aphids during feeding. These data support the hypothesis that smallRNAs play an important role in host plant responses to phloem feeding insects, in both resistant and susceptible interactions.

Financial support: USDA-AFRI

EFFECTS OF STARVATION ON PROBING BEHAVIOR OF THE ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI*

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Some hemipterans undergo periods of starvation in the absence of host plants or during migratory flights, which may result in changes in physiology and feeding behavior. *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), an important vector of phloem-restrited bacteria associated with citrus huanglongbing (HLB), is an olygophagous psyllid with host range restricted to a few genera in Rutaceae, and may experience starvation if adults move long distances searching for host plants or citrus groves. Although *D. citri* is a typical phloem feeder, a previous study indicated that starved adults often ingest xylem sap. By using the electrical penetration graph (EPG) technique, we investigated changes in probing behavior of this psyllid vector after increasing periods of starvation (0, 6, 12 and 24 h). For each starvation treatment, 15 adult females (4-6 days old) were recorded for 5 h on seedlings of *Citrus sinensis* (L.) Osbeck using a Giga-8 DC-EPG monitor. Except for a reduction in the number of probes in the first hour of probing, most starvation effects were observed on EPG parameters related to the xylem and phloem phases of probing. The percentage of insects that performed waveform G (putative xylem ingestion) increased from 28.6 (non-starved) to 53.3, 93.3 and 80% when starved for 6, 12 and 24 h, respectively. The number of G waveform events was significantly higher in insects starved for at least 12 h, whereas the mean duration of G per event increased in all starvation treatments. On the other hand, non-starved insects started phloem ingestion (waveform E2) faster than starved ones. Some individuals did not reach the phloem phase during the 5-h recording time after starved for 12 h (26.7%) or 24 h (13.4%), whereas all non-starved ones did. Interestingly, mean durations of waveforms D (phloem contact) and E1 (waveform that precedes phloem ingestion) were significantly reduced in starved insects, regardless of the starvation period. Overall, the data indicate that long starvation periods (≥12 h) induce most *D. citri* adults to ingest xylem sap before the phloem phase, delaying the onset of phloem ingestion. These starvation-mediated changes in probing behavior, if common in migrating psyllids, may have implications on the efficiency of systemic insecticides that are transported through the xylem vessels.

Financial support: Citrus Research Development Foundation (Florida/USA), CNPq/Brazil, Fundecitrus/Brazil

FEEDING BEHAVIOUR AND PERFORMANCE STUDIES ON DIFFERENT POPULATIONS OF THE BLACK CURRANT - LETTUCE APHID *NASONOVIA RIBISNIGRI* ON RESISTANT AND SUSCEPTIBLE LETTUCE CULTIVARS

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The black currant-lettuce aphid, *Nasonovia ribisnigri* (Mosely), is an economically important pest of lettuce, *Lactuca sativa* L. Lettuce can get seriously damaged by high numbers of this aphid species, causing deformation of the head, changing leaf colour and reduction of vigour in seedlings. Although small numbers of aphids have no effect on yield, the presence of living aphids is a cosmetic problem, making the lettuce unmarketable. Additionally *N. ribisnigri* is acting as a vector of viruses, including Cucumber mosaic virus and Lettuce mosaic virus. The control of the lettuce aphid in cultivated lettuce is largely based on genetic host plant resistance, considered to be the most desirable control measure for this aphid. Host plant resistance is based on a single gene, the *Nr*-gene, introgressed from the wild lettuce species *Lactuca virosa* L. which provides absolute resistance against *N. ribisnigri*. The *Nr*-gene has not yet been cloned and the resistance mechanism is not known. Moreover, *Nasonovia* populations insensitive to the *Nr*-based resistance in lettuce have emerged in several locations in Europe since 2007. The objective of this project is to unravel the resistance mechanism of the *Nr*-gene in lettuce by a combined metabolomics / proteomics / transcriptomics approach of phloem composition studies, in concert with detailed behavioural and performance studies on the aphid. The identification of the chemical basis of host-plant resistance will allow plant breeders to accelerate their breeding programmes. Using the EPG-technique, the penetration and feeding behaviour of 5 different *Nasonovia ribisnigri* populations (biotype 0, biotype 1 from Germany, biotype 1 from Paris, biotype 1 from Perpignan and biotype 1 from Belgium) were studied on the resistant cultivar Corbana and the susceptible cultivar Pinokkio. In the survival tests the mortality and larval development were studied. Additionally, reproduction and colony development tests were performed. Biotype 0 aphids showed a significant reduction in phloem sap intake on Corbana compared to Pinokkio and almost all aphids died on Corbana. The few that were able to survive and developed into adults on Corbana, took 14 days to develop into adults, compared to 8 days on Pinokkio. From the biotype 1 populations the populations from Belgium and Perpignan showed a significant reduction in phloem sap intake on Corbana compared to Pinokkio, whereas the German and Paris population did not show a significant reduction of E2. The German population seems to have the least difficulties to feed on Corbana, while the Perpignan population has most difficulties to feed on Corbana. This trend was also observed in the survival experiment in which aphids from the German population showed the highest survival and aphids from the Perpignan population showed the lowest survival. Overall the aphids of the biotype 1 populations took 2 to 4 days longer to develop into adults on Corbana compared to Pinokkio.

RESISTANCE TO CABBAGE WHITEFLY IN *BRASSICA OLERACEA*

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The cabbage whitefly (*Aleyrodes proletella*) is a phloem-feeding insect that is becoming more and more of a problem in Western Europe. Especially Brussels sprouts, kale and Savoy cabbage can be heavily infested. Besides causing cosmetic damage, whiteflies excrete a sugary substance (honeydew) that allows the growth of sooty mould. Both types of damage reduce the marketability of the crop. The use of pesticides is hazardous to the environment and usually not very effective as whiteflies feed on the underside of leaves. Breeding for resistance would be a sustainable alternative. We have identified a white cabbage (*Brassica oleracea* var. *capitata*) variety with high levels of resistance to the cabbage whitefly and also a variety that is very susceptible to this insect (Broekgaarden et al. 2010, J. Exp. Bot., 61: 807). The resistance is seen both under field conditions and under controlled conditions in the greenhouse, in choice and non-choice experiments. The resistance is dependent on the growth phase of plant, older plants show a higher level of resistance than young plants. Detailed studies on the interaction between plant and insect show that the resistance is most likely phloem based.

CHARACTERIZATION OF CORN LEAFHOPPER, *DALBULUS MAIDIS* DELONG AND WOLCOTT ELECTRICAL PENETRATION GRAPH WAVEFORMS

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The stylet probing activities of the leafhopper, *D. maidis*, a vector of corn stunt spiroplasma, *Spiroplasma kunkelii*, were studied by electrical penetration graph technology. Six distinct waveforms were characterized and correlated with major probing activities of *D*. *maidis* via transmission of corn stunt spiroplasma and excretion of honeydew as markers. Major waveforms comprise stylet pathway (waveform 1), active ingestion in non-sieve elements (waveform 2), nonvascular probing (waveform 3), phloem contact (waveform 4, the X wave), phloem ingestion (waveform 5) and oviposition (waveform 6). Our results support most previous findings for this species, and also indicate that some waveforms (2, 4 and 5) are related to biopotentials generated during probing, as was previously found for other hemipteran species. The most important finding from this work is that *D. maidis* ingests from phloem sieve elements more frequently and for longer durations than seen in previous research, probably due to longer observation periods used in this study. This work provides basic information relevant to the understanding of probing behavior of *D*. *maidis* and to the characterization of potential sources of insect-resistant maize.

NEW SOURCES OF RESISTANCE TO LETTUCE APHIDS IN *LACTUCA* SPP.

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The use of resistant cultivars is one of the best ways to protect lettuce from aphid pests. At the moment, there are cultivars available with nearly total resistance to *Nasonovia ribisnigri* biotype Nr:0 (based on the *Nr* gene) and partial resistance to *Macrosiphum euphorbiae*. Nevertheless, a new biotype of *N. ribisnigri* (Nr:1) able to overcome the resistance based on the Nr gene is expanding around Europe and has become a major threat of lettuce. In the present work we report the presence of this new biotype in south-eastern Spain, a major lettuce-producing region. Furthermore, a pool of 264 germplasm accessions from public germplasm banks belonging to *Lactuca* genus was tested on a field assay to search for new resistance sources to the biotype Nr:0 of *N. ribisnigri*. The most promising accessions were retested in the laboratory to characterize the resistance by means of free-choice and antibiosis assays against biotypes Nr:0 and Nr:1 of *N. ribisnigri* and against a clone of *M. euphorbiae*. Three accessions of *L. virosa* showed resistance against the target aphid species and could be of interest to ongoing breeding programs. The accessions CS056, CS068 and CS020 were nearly total resistant to Nr:0 and partially resistant to *M. euphorbiae,* but only CS056 and CS020 were partially resistant to the biotype Nr:1. The accession CS048, in spite of not being resistant to *N. ribisnigri* biotypes, showed a near total resistance to *M. euphorbiae.* Later, 42 additional accessions of *L. virosa* from the CGN germplasm bank were tested in laboratory for their resistance to *N. ribisnigri*. Eight accessions showed a nearly total resistance to Nr:0 and among them one (CS298) showed a very good resistance profile to Nr:1 in free-choice antixenosis and antibiosis assays.

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RESISTANCE OF *BRASSICA OLERACEA VAR. ACEPHALA* TO *BREVICORYNE BRASSICAE* (LINNAEUS, 1758) (HEMIPTERA: APHIDIDAE)

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Among the pests of crucifers, there is the aphid *Brevicoryne brassicae* L. collard green (Hemiptera: Aphididae) with great economic importance due to the sucking sap and virus transmission. Was evaluated under laboratory conditions (T: 25 ± 2 º C RH 70% and photophase: 12h) the attractiveness of *B. brassicae* for seven genotypes of collard green: “Manteiga de São Roque I-1812”, “Comum”, “Hortolândia”, “Manteiga de Tupi”; “Crespa I-918”, besides the commercial variety “Manteiga da Geórgia” and an introduction of the Municipality of Arthur Nogueira. Circular arenas were made of polystyrene (36 cm diameter), around which were randomly allocated to the leaves of the genotypes studied, keeping the petiole immersed in the same containers with water in order to maintain the turgidity of the leaves. In the center of the arena were released 35 adult wingless aphid, which were quantified in each genotype after 15, 30 and 60 minutes. The experimental design was randomized blocks with eight replications. The results show that the variety “Manteiga da Geórgia” was the least sought after by aphids, while the variety “Comum”, and the Introduction of the municipality of Arthur Nogueira were more attractive.

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ANALYSIS OF ELECTRICAL PENETRATION GRAPH DATA: WHAT TO DO WITH ARTIFICIALLY TERMINATED EVENTS?

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Observing the durations of hemipteran feeding behaviors via Electrical Penetration Graph (EPG) results in situations where the duration of the last behavior is not ended by the insect under observation, but by the experimenter. These are artificially terminated events. In data analysis, one must choose whether to retain these observations or discard them. This study sought to gain a better understanding of the consequences of this choice. Raw data from two EPG experiments were used, one with cotton-melon aphid (*Aphis gossypii*), and the other with Asian Citrus Psyllid (*Diaphorina citri*), to study the consequences of one's choice under two conditions frequently encountered in analysis of EPG data: 1) data are randomly distributed about some average value, and 2) the special case where each observation of a specific waveform event lasts longer than the previous observation (i.e. monotonically increasing). This situation was predicted in the literature and was observed in the raw data. Simulations in Excel were used to generate three data sets that differed only as follows: 1) no artificially terminated event; 2) last observation artificially terminated; 3) last observation deleted. Analysis found that, in the general case, it is best to delete the artificially terminated event before further analysis. However, artificially terminated events should be retained in many cases if durations are monotonically increasing. Equations and graphs are provided to help decide in which cases one is better off retaining artificially terminated events based on how one's choice biases the estimated mean and standard deviation.

INFLUENCE OF PLANT EXTRACTS ON REPELLENCE AND OVIPOSITIONAL PREFERENCE OF *BEMISIA TABACI* (GENN.) BIOTYPE B (HEMIPTERA: ALEYRODIDAE) FOR TOMATO

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Whitefly is one of the most harmful pests that attack tomato crops, mainly for extracting large quantities of phloem sap and transmitting the virus. Aiming to explore alternative method for controlling this insect, the present study evaluated the repellence and oviposition deterrence of 13 botanical extracts (3%), including water and Thiametoxam (negative and positive controls) under greenhouse conditions. Tomato plants (35 days after emergence) were used in the research. Before infestation, the plants were sprayed with 15 treatments. After 15 minutes of application, the potted plants were randomly distributed in a circle inside the cages (2.5 x 3.0 x 2.5 m) with 1500 adult whiteflies (50 couples per treatment). The repellence was assessed with a mirror. Observed the total number of insects present in three leaflets per plant previously marked (upper, middle and lower) with 24 and 48 hours after release of insects; 72 hours after infestation, the deterrence was evaluated in three leaflets per plant previously marked (upper, middle and lower). The plants were then removed from the cage and the number of eggs per leaflets was counted with the aid of a microscope-stereoscope. The experiment was conduced in a randomized complete block design with 15 treatments and six replicates. The data were submitted to ANOVA and the mean values were compared by using the Tukey test ($P \leq 0.05$). There was no difference among treatments for both parameters, however, plants sprayed with the extracts of *Trichilia pallida, Trichilia casaretti* and *Chenopodium ambrosioides* showed a lower number of insects attracted and a number of eggs to the bottom of witnesses.

INFLUENCE OF A FATTY ACID DESATURASE ON PLANT-APHID INTERACTIONS

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We report here that disruption of function of the ω-3-fatty acid desaturase FAD7 in tomato reduces host suitability for aphids. The *spr2* mutation, which eliminates function of FAD7 and dramatically decreases the foliar content of trienoic fatty acids, also causes a decrease in host preference, survival and fecundity of the potato aphid, *Macrosiphum euphorbiae*. Monitoring of aphid feeding behavior by the direct-current electrical penetration graph (DC-EPG) technique indicates that ingestion from the phloem is inhibited on *spr2* plants, suggesting that resistance in this mutant may be due to factors localized in the phloem. Aphid resistance in *spr2* also requires the plant hormone salicylic acid, and NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 (NPR1), a positive regulator of many salicylate-dependant defenses. These results suggest that fatty acid desaturase activity in plants negatively regulates phloem-limited salicylate-dependant defenses against aphids. The potential implications of plant fatty acid profiles for aphid nutrition will also be discussed.

A FACILITATED GLUCOSE/FRUCTOSE TRANSPORTER OF THE PHLOEM-SAP FEEDING INSECT, THE BROWN PLANTHOPPER, *NILAPARVATA LUGENS*

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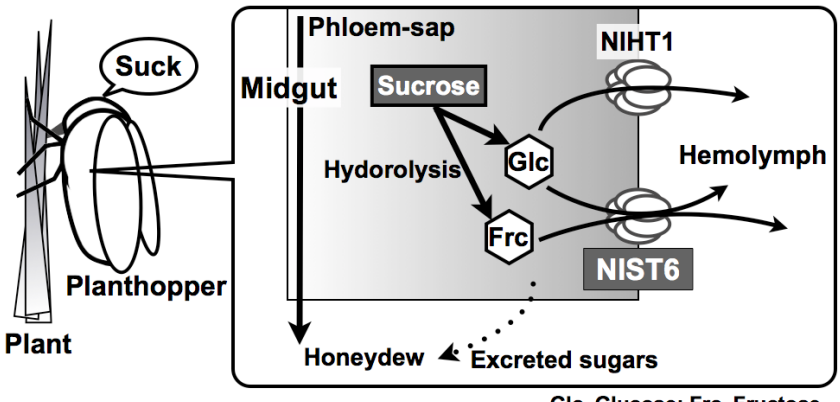
The brown planthopper (BPH), *Nilaparvata lugens*, attacks rice plants and sucks their phloem-sap, which contains large amounts of sugars. The main sugar component of the phloem-sap is sucrose, a disaccharide composed of glucose and fructose. Sugars hydrolyzed to monosaccharides appear to be incorporated into the planthopper body across the sugar transporters in the midgut. Recently, a facilitated glucose transporter gene expressed in the midgut, *NlHT1*, has been identified in BPH (Price et al., 2007). NlHT1 did not function for fructose uptake, therefore, sugar transporter for fructose uptake remains unknown in planthoppers. A total of 93 expressed sequence tags (ESTs) for putative sugar transporter genes were obtained from a BPH cDNA EST database (http://bphest.dna.affrc.go.jp/), and 18 putative sugar transporter genes (*Nlst1-18*) were identified. The most abundantly expressed gene was *NlHT1 (Nlst1)*. *Nlst1, 4, 6, 9, 12, 16*, and *18* were highly expressed in the midgut. It is difficult to identify substrates of sugar transporters based on their primary amino acid sequences. Therefore, the functional analyses of NlSTs were performed using the *Xenopus* oocyte expression system. This showed that NlST6 was a facilitative glucose/fructose transporter that mediates sugar uptake from rice phloem-sap in the BPH midgut in a manner similar to NlST1. Kinetic analyses revealed that NlST6 was more effective for fructose transport than for glucose transport. The facilitative sugar transporter move sugars along gradients from regions of high concentration to those of lower concentration. BPH obtains sugars as a nutrient sources from the phloem-sap and carries them to the hemolymph by driven gradients across the transporter. This is the first report of the identification of the transporter responsible for incorporating fructose in planthoppers.



Sugar flow in planthopper.
Sucrose in rice phloem-sap is hydrolyzed into glucose and fructose in the midgut. NlST6 transported the glucose and fructose into the hemolymph.

MICROORGANISMS FROM APHID HONEYDEW ATTRACT AND ENHANCE THE EFFICACY OF NATURAL ENEMIES

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Aphids are one of the most serious pests of crops worldwide, causing major yield and economic losses. To control aphids, natural enemies could be an option but their efficacy is sometimes limited by their dispersal in natural environment. Here, we report the first isolation of a bacterium from the pea aphid *Acyrthosiphon pisum* honeydew, *Staphylococcus sciuri*, which acts as a kairomone enhancing the efficiency of aphid natural enemies. Our findings represent the first case of a host-associated bacterium driving prey location and ovipositional preference for the natural enemy. We show that this bacterium plays a key role in tritrophic interactions because it is the direct source of volatiles used to locate prey. Some specific semiochemicals produced by *S. sciuri* were also identified as significant attractants and ovipositional stimulants. The use of this host-associated bacterium could certainly provide a novel approach to control aphids in field and greenhouse systems.

THE DISRUPTION OF PRIMARY ENDOSYMBIONTS OF *MYZUS PERSICAE* INFLUENCES THE APHID FEEDING BEHAVIOR

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Myzus persicae (Sulzer), like all aphids, associates with the endosymbiotic bacteria *Buchnera aphidicola* which is localized in the aphid hemocoele. Although the accepted function of *B. aphidicola* is to provide nutrients to complete the aphids diet (essential amino acid, vitamins, etc.) there are evidences of other roles. Our hypothesis is that this endosymbiont has an active role in the plant-aphid interaction in relation to host acceptance. In this work we analyzed how the feeding behavior of *M. persicae* is affected by antibiotic treatment against *B. aphidicola*. Young adults of *M. persicae* were treated for 7 days with the antibiotic chlorotetracycline through artificial diets to disrupt *B. aphidicola* (aposymbiotic aphids) and their feeding behavior on radish plants (*Raphanus sativa*) was monitored with electrical penetration graph (EPG) technique. Two sets of controls were used, 1) aphids reared on radish until adulthood and then fed for 7 days on artificial diets without the antibiotic, and 2) aphids reared on radish until the EPG monitoring day. We found that aposymbiotic aphids have stylet pathway constrains, perform less cell punctures and need more time to start the phloem activities than the aphids fed on normal diet or in radish. Also salivation and ingestion of phloem sap were impeded, resulting in a significantly reduced number of aphids capable of host acceptance. Although the artificial diet has contributed to some odd probing behavior, e.g. increased xylem contact and longer time to start phloem activities, in all cases the effect was significantly increased by the antibiotic treatment.

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MOLECULAR EVIDENCE FOR THE PRESENCE OF *BEMISIA TABACI* BELONGING TO THE MIDDLE EAST-ASIA MINOR 1 AND NEW WORLD SPECIES IN BRAZIL

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Bemisia tabaci is one of the most important insect pests, being vector of the plant virus begomoviruses and causes serious problems in many countries, including Brazil. Based on the mitochondrial cytochrome oxidase I (mtCOI) sequence, the phylogenetic relationships from populations of *B. tabaci* collected from different hosts and locations in São Paulo and Mato Grosso State, Brazil were analyzed. According to the recent classification of *B. tabaci*, the most part of the specimens collected in Brazil belongs to the Middle East- Asia Minor 1 specie, which includes biotypes B and B2. But three specimens collected from *Euphorbia heterophylla*, *Xanthium cavanillesii* and *Glycine max* (soya) respectively, were classified in the New World group/specie and showed higher nucleotide identity with *B. tabaci* from Colombia (accession number AJ550167 and AJ550168, A biotype). The different species could be found colonizing the same soya plant in commercial area of Mato Grosso, indicating the co-existence of them in Brazil. By RFLP, these species could be easy differentiated using *Thru* I and *Taq I* enzymes. Mediterranean specimens could not be found in Brazil. As far as we know this is the first molecular evidence for the presence of the New World specie in Brazil.

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PHAGODETERRENT EFFECT OF *TRICHILIA PALLIDA* EXTRACT AGAINST *RHOPALOSIPHUM MAIDIS*

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Intending to find new botanical pesticides showing repellent action on aphids, we used Electrical Penetration Graphic Technique to assess the phagodeterrent effect of a hexanic sub-fraction of *Trichilia pallida* extract against *Rhopalosiphum maidis*. Thus, each individual aphid was starved for 1 hour and placed on the youngest leaf of the plants 30 min previously treated with 0.5 ml in an extract concentration of 2% and other plants sprayed with water plus adhesive spreading were used as control. Both treated and control treatments had 15 replicates, and the probing behavior was recorded for 5 hours. The parameters evaluated were: non-probing; pathway activities; number of pathway activities; phloem salivation; number of phloem salivation; phloem ingestion; number of phloem salivation; xylem ingestion; number of xylem ingestion; intracellular punctures; number of intracellular punctures; time to first intracellular puncture; total time of penetration; time to reach the first phloem ingestion. Analyzing the averages of the variables, we observed a feeding behavior alteration of aphids on treated plants with the hexanic fraction when compared to feeding behavior of aphids on untreated plants. The parameters: time of non-penetration, intracellular puncture, number of intracellular punctures and time to reach the first phloem ingestion showed that the insects had more difficulty to feed on plants sprayed with hexanic extract. Besides that, the parameters phloem salivation and number of phloem salivation were higher on the treatment with hexanic than untreated treatment. Another characteristic of insects on untreated plants was the non-success of keeping a normal feeding because the phloem salivation was interrupted before the phloem ingestion beginning.

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PARTICULARITIES AND VARIABILITY ON MEALYBUG PROBING BEHAVIOUR

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Stylet insertion seems to be crucial to understand sucking-insect plant interactions. Preliminary tests showed great variability when monitoring mealybugs specially those events related to phloem. Thus, long recording were necessary (at least 16 hours) to obtain as different probing phases. So, the mealybug probing behaviour was studied by means of the Electrical Penetration Graphs (EPG) in several insect/plant combinations to identify particularities and variability. The results showed that mostly cell punctures shows only two phases, equivalent to phases II-1 and II-2 of aphids, which means that cell sap ingestion would be absent or highly reduced. Whether its absence is responsible for failing non-persistent virus (NPV) transmission is an open question since mealybugs have not been reported as NPV vectors**.** Waveform "N", a new described waveform and apparently restricted to mealybugs, is a highly variable pattern, recorded at extracell level with stylets located in mesophyll tissue and occasionally can last for several hours. E_1 duration is rather short (few minutes) and frequently shows weak peaks, resulting in an ambiguous shift to E_2 pattern. Mealybug probing behaviour presented also high variability depending of the mealybug/plant combination. For example, phloem phase is reached earlier and last longer in *Planococcus citri* on coffee and *Phenacoccus solenopsis* on Spanish needle, as compared with *Pl. citri* on citrus and *Dysmicoccus brevipes* on cell cultured pineapple plants.

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ADAXIAL VS. ABAXIAL DIFFERENCES IN PREFERENCE AND PROBING BEHAVIOUR OF *BEMISIA TABACI* ON ACYLSUCROSE-PRODUCING TOMATO

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The whitefly *Bemisia tabaci* (Genn.) is widely distributed in warm and temperate regions throughout the world. Previous studies conducted with the wild tomato *Solanum pimpinellifolium* L. accession TO-937 and the introgression line ABL 14-8, indicated that both genotypes were resistant to *B. tabaci*, the vector of tomato yellow leaf curl disease (TYLCD). This resistance was based on presence of type IV glandular trichomes and the production of acylsucroses, mainly located on the abaxial leaf surface. The influence of the adaxial and abaxial tomato leaf surface of the nearly-isogenic lines 'Moneymaker' and ABL 14-8 (without and with type IV glandular trichomes, respectively) on *B. tabaci* settling (under choice and non-choice conditions) as well as the probing and feeding behavior was studied on plants at the 4-leaf growth stage, prior to full resistance expression, and at the 10-leaf growth stage, when the resistant traits were present. . Experiments were carried-out on the third youngest leaf of test plants. Significant differences were observed at the 4-leaf stage on the adaxial leaf surface under non-choice conditions, where the mean number of adult whiteflies per leaflet was higher on ABL 14-8. At the 10-leaf stage, significantly lower whitefly numbers were counted on the abaxial leaf side of ABL 14-8 than on 'Moneymaker' under both free-choice and no-choice tests. The opposite was observed on the adaxial surfaces in which significantly higher counts of *B. tabaci* occurred in ABL 14-8 than in 'Moneymaker'. These results indicate that *B. tabaci* avoids settling on the abaxial side of the leaflet of ABL 14-8 when the plants are at the 10-leaf stage. Significant differences were observed for the feeding behavior of *B. tabaci* on both leaflet sides of 'Moneymarker' suggesting preference of whiteflies to feed on the abaxial surface with a higher number of sustained feeding ingestion events from the phloem sieve elements. However, for ABL 14-8 in which type IV glandular trichomes and acylsucrose accumulation are present mainly on the abaxial surface of leaves, differences in feeding behavior indicated a preference to settle and probe on the adaxial than on the abaxial side of the leaf.

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HOST PRECONDITIONING IN MEALYBUGS (PSEUDOCOCCIDAE)

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Mealybug probing behaviour studied by means of Electrical Penetration Graphs (EPG) showed that either they do not reach the phloem or this is delayed to 9 to 20 hours. However, the phloem phase was reached earlier and more frequently when the mealybugs were reared on the same host used for monitoring. This result suggests the presence of host preconditioning similar to that found on some aphids. This study aimed to detect the effect of the previous experience on mealybugs through choice tests, development studies and probing behaviour monitoring. The citrus mealybug, *Planococcus citri* (Risso) reared on squash, citrus and coffee was used in all experiments. The choice test between coffee and citrus, showed that mealybugs reared on coffee showed a high preference to settle on coffee. When the source plant was citrus the mealybugs showed a trend, even not significant, to select citrus over coffee. Mealybugs taken from a squash culture did not show any preference neither for coffee or citrus. Insects transferred from squash to coffee or citrus, and from coffee to citrus, showed a significant reduction in the number of insects presenting the phloem phase and an increasing of the non-probing time. The proportion of mealybugs with phloem phase was not affected by transferring insects from citrus to coffee but the phloem phase was reduced. Transferring mealybugs, either as eggs or nymphs, from any host to coffee or citrus did not modify the development time, fecundity or mortality. Thus, even showing some preference for the source plant both, coffee and citrus, were good substrates for the mealybug development. These results indicate changes in responses to plants depending to the previous experience and it is necessary to consider the parents´ culture host species when working on mealybug behaviour or physiology.

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CHARACTERIZING FEEDING BEHAVIOR OF *ACYRTHOSIPHON PISUM* CLONES ON HOST AND NON-HOST PLANT SPECIES BY THE ELECTRICAL PENETRATION GRAPH (EPG) TECHNIQUE

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The pea aphid, *Acyrthosiphon pisum* (HARRIS), encompasses distinct races differentially specialized on plant species of the Leguminosae. This ecological specialization, which leads to the formation of what is known as “host races”, can be considered as one step towards sympatric speciation. Host fidelity (the tendency to feed and reproduce on a particular host) leading to assortative mating seems to be an important mechanism that reduces gene flow between host races. Our study aims to localize and identify plant factors which influence host fidelity and hence host race formation in *A. pisum.* We conducted a broad comparative study on several *A. pisum* clones collected from different legume host plants in France and the United Kingdom. Firstly, we characterized the performance of nine *A. pisum* clones on six legume plant species, and showed different degrees of specialization to potential host plant species. Secondly, we selected six aphid clones and monitored their feeding behavior on four plant species by the Electrical Penetration Graph (EPG) technique. The differences we found in feeding behavior can explain the different degrees of host plant specialization. Moreover we obtained several hints for the localization of plant factors influencing aphid feeding behavior in different plant tissues.

GENDER DIFFERENCES AND EFFECT OF PHOTOPHASE ON ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI* KUWAYAMA) FEEDING BEHAVIOR

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Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), thought to be primarily a phloem-feeding insect, transmits the Huanglongbing pathogen, *Candidatus* Liberibacter asiaticus. Because this bacterium is restricted to the phloem and bacterial transmission is the result of psyllid feeding, investigation of the basic feeding behavior of *D. citri* is needed. In this study, an electrical penetration graph (EPG) monitor was used to: 1) determine whether gender-based differences in feeding behavior exist for *D. citri* and 2) examine the effects of photophase on *D. citri* feeding activities. In the first experiment, investigating gender-based differences in *D. citri* feeding behavior, overall, the number of male *D. citri* reaching the phloem was 20% higher compared to females. However, the mean duration of phloem ingestion (waveform E2) per insect was significantly higher for female *D. citri* compared to males. Analysis within treatments (gender) showed that, despite being considered a phloem feeder, the duration of xylem ingestion (waveform G) and phloem ingestion (waveform E2) were not significantly different. Prominent xylem ingestion was probably not caused by desiccation due to trauma, because insects were not anesthetized, starved or excessively handled during wiring. In the second experiment, examining the effects of photophase on *D. citri* feeding, non-probing activities (waveform z and np), phloem penetration and salivation (waveform D and E1, respectively), and xylem ingestion (waveform G) were generally longer in duration per insect during the light photophase. However, stylet pathway activities (waveform C) and phloem ingestion (waveform E2) were longer in duration during the dark photophase. Within-treatment analysis indicated some effects of photophase on xylem ingestion (waveform G) and phloem ingestion (waveform E2). Thus, results suggest that gender and photophase have an influence on *D. citri* feeding behavior, and are important variables that could affect the outcome of experiments investigating the transmission of *Candidatus* Liberibacter asiaticus by *D. citri*.

ANTIBIOSIS AND NON-PREFERENCE FOR FEEDING ON *BEMISIA TABACI* BIOTYPE B IN SOYBEAN GENOTYPES

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The whitefly, *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) has become an important insect to the soybean crop, with potential to reach the status of key pest. The use of resistant genotypes, which affect the biological development of this insect, is considered one of the most important methods to control this pest. This study aimed to detect the occurrence of non-preference for feeding and / or antibiosis on *B. tabaci* biotype B in nine genotypes of soybean, *Glycine max*, under laboratory conditions, to detect possible mechanisms of resistance against this insect attack. Thus, nine genotypes were planted in pots, remaining free from the whitefly infestation up to 30 days of age. After that, three leaflets per plant were individualized, mating cages made of cheesecloth and releasing inside each of them 25 pairs of the insect. After 24 hours of infestation, the insects were removed and the plants carried to the laboratory. After examining the leaflets, an area with 30 eggs on the abaxial surface of each of them was isolated to biological evaluations. Each leaflet represented a repetition, in a total of three per genotype in an entire randomized design. The evaluations were daily and at the same time, observing the following biological parameters: incubation period, duration and mortality of nymphal instars, developmental period from egg to adult and larval viability. Based on these results, it was found that genotype Conquista prolonged the duration of the second instar nymph of *B. tabaci* biotype B, the same was verified with the BRS-242 RR genotype in the 3rd instar and also with IAC IAC-19 and PL1 in the 4th instar, indicating the occurrence of small levels of non-preference for feeding and / or antibiosis . Also based on the biological results obtained in laboratory , it was noticed that the IAC- PL1 genotype extended the total cycle of the nymphal development, suggesting the occurrence of non-preference level for feeding and / or antibiosis. There were no significant differences related to the materials regarding the incubation period, 1st instar, mortality and viability.

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RELIABILITY OF EPGs IN ANSWERING PLANT-INSECT QUESTIONS

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Studies on interactions between plants and aphids – and other insects with piercing-sucking mouthparts – are now rather wide spread using the electrical penetration graph (EPG) technique. Some reflections and prospects on this method and how to use its results may be appropriate therefore. The technique has shown to be a valuable bioassay to predict what occurs under 'natural conditions'. However, we mostly use potted plants grown in greenhouses while insects are tethered and put on plants without normal host location and selection and without free take off possibilities. EPGs are recorded in half dark laboratories without normal daylight periods. So, we seem to assume that all this doesn't matter or if it would, our control plants or insects are dealing with the same conditions. In summary, these are EPG experiment design aspects.
Another aspect of concern is that after EPG recording and waveform analysis, the data are processed into EPG variables (or parameters), which are used as indicators to support answers to our research questions. In scientific journals and meetings we use these variables to communicate. However, it seems that different authors are using the same variable name but use a different definition and calculation, which causes miscommunication. EPG experimental design aspects and EPG variables will be discussed and examples will be presented of design errors and why variables should be standardised.

TRANSPORT OF NUTRIENTS AND DEFENSIVE COMPOUNDS IN THE PHLOEM

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Several classes of defensive, secondary compounds are found in the phloem and may be transported along with carbohydrates and other nutrients. These defensive compounds can potentially protect the phloem against aphids and other phloem feeders, as well as sink tissues. However, distinguishing between molecules that are authentically mobile and those that are localized in adjacent cell types is a challenge due to the complex nature of the phloem, as well as the strong propensity of sieve elements to violently displace their contents when severed. Several methods of phloem sampling will be discussed, including phloem bleeding, EDTA-facilitated exudation, and the use of severed aphid stylets. These methods will be discussed in the context of carbohydrate and iridoid glycoside translocation.

PHLOEM-FEEDERS VERSUS PHLOEM SEALING MECHANISMS

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Phloem is one of the two main transport tissues in plants and is responsible for translocation of most of the organic nutrients (e.g., carbohydrates, amino acids, proteins) that are transported throughout the plant. Many insects, almost exclusively hemipterans, exploit phloem sap as their primary source of nutrition. Two of the major advantages of specializing on phloem sap are 1) the high nutritional content of phloem sap, and 2) phloem sieve tubes (the actual transport conduit in phloem) function as a pipeline with a continuous flow of phloem sap; thus once a sieve tube is "tapped" it can provide an almost inexhaustible supply of sap, allowing a phloem-specialist to feed from the same sieve tube for hours, or in the case of whitefly nymphs, an entire nymphal instar. However, just the same as animals have clotting and coagulation mechanisms to prevent loss of blood when their vascular system is damaged, plants have analogous mechanisms that seal damaged sieve tubes to prevent the loss of phloem sap. Consequently, in order to successfully exploit the pipeline properties of sieve tubes, all phloem-specialists need to have counter-measures that either prevent triggering the sealing response and/or to reverse the sealing response if it occurs. Phloem-specialists such as aphids and whiteflies inject considerable amounts of watery saliva into sieve tubes the function of which has been widely hypothesized to prevent or reverse the sieve tube sealing response. Recent evidence *in vitro* indicates that watery saliva of aphids is capable of reversing at least one of the sealing mechanisms in legume phloem: a protein plug that arises from a proteinaceous inclusion body, the forisome, that is characteristic of legume sieve tubes. This presentation describes the early stages of a 3-year project to determine the role of saliva or other counter-measures in overcoming the sieve tube sealing response using *in vivo* methods. Ingestion of phloem sap from sieve tubes by aphids and whiteflies is always preceded by a period of salivation into the sieve tube. This salivation period has frequently been postulated to be a response to reverse sieve tube sealing that presumably occurs when the sieve tube is pierced. This hypothesis was tested with the pea aphid, *Acyrthosiphon pisum*, feeding of faba bean, *Vicia faba*. The feeding site of the aphid was cryofixed shortly after penetration of the sieve tube near the beginning of the salivation phase. This fixed the tissue almost instantaneously and the stylets usually remained in the sieve tube; consequently, the specific sieve element (the cells making up the sieve tubes) that was pierced could be identified and the state of the forisome (either in its compact non-plugging phase or in its dispersed plugging phase) in that sieve element could be determined at this exact point in time using confocal microscopy. No evidence of forisome plugging of sieve tubes was detected. Pea aphids generally precede "phloem-phase" behavior (salivation into and ingestion of phloem sap from sieve tubes) by a repeating series of penetrations of the sieve element. In order to test the hypothesis that these "pre-phloem phase" penetrations resulted in forisome plugging that was eventually reversed

by the time phloem phase began, the cryofixation technique was used to instantaneously fix the phloem and stylets so that the state of the forisome could be examined during "pre-phloem phase" sieve tube penetrations. Again, no evidence of forisome plugging of sieve tubes was detected. Consequently, it appears that initial penetration of the sieve elements does not trigger a phloem-sealing response at least in the pea aphid - faba bean system, and that the function of the initial salivation into sieve elements immediately following penetration is something other than reversing a sealing response. These results also generate the question: why/how does stylet penetration of a sieve element not trigger a sealing response?

X-WAVE FILES: STEREOTYPICAL VASCULAR CELL-PROBING ACTIVITIES REVEALED BY EPG

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Vascular sap-ingesting hemipterans must overcome a number of host plant defense mechanisms to establish the strong stylet connection necessary to sustain ingestion from phloem sieve elements or xylem tracheary elements. Using electrical penetration graph (EPG) technology, many hemipteran species representing several taxa have been recorded. Resulting waveforms have been identified, characterized and correlated using histology and biochemical tests to determine stylet location within plants. Historically, three waveform types have been associated with stylet activities in vascular cells, i.e. phloem salivation, sustained phloem or xylem ingestion, and another waveform that usually precedes the first two. The latter waveform has been assigned a myriad of behavioral interpretations and names, but its original designation has been the most enduring: the X-wave. By definition, the X-wave is a waveform that is stereotypical in appearance, comprising repeated phrases, and is produced upon first stylet contact with phloem or xylem. The X-wave is easily recognized in all types of EPG outputs, and X-waves of related hemipteran species share common characteristics. Here, X-waves from three sheath-feeding leafhoppers, two aphids, one psyllid, and one true bug, are presented, analyzed, and discussed. In addition, a compilation of X-waves and their interpretations from the literature are considered. Finally, it is proposed that X-waves represent stereotypical vascular-cell-acceptance and conditioning behaviors for each species, and that X-waves may be used as quantifiable taxonomic characters for phylogenetic analyses. Colleagues are invited to contribute EPG-X-waves from their study species, and to collaborate on future research to test this hypothesis.

ATTRACTIVENESS AND NON-PREFERENCE FOR OVIPOSITION OF *BEMISIA TABACI* (GENN.) BIOTYPE B (HEMIPTERA: ALEYRODIDAE) IN SQUASH GENOTYPES

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The management of *Bemisia tabaci* biotype B has become a challenge for farmers, since it has a high ability to develop resistance to all classes of insecticides. An alternative is the use of resistant plants as a management practice. The objective of this study was to evaluate 20 genotypes of squash (*Cucurbita* spp.) regarding the attractiveness and non-preference for oviposition of *B. tabaci* biotype B, in a free choice greenhouse experiment. A randomized block design with 20 treatments (genotypes) and 10 repetitions was used, totaling 200 plots. Each plot consisted of one pot containing two squash plants about 18 days old. Under free choice conditions, the pots containing squash genotypes were distributed randomly in a circle inside net cages (2.0 x 2.0 x 2.5 m). A ratio of 50 whitefly couples per pot was release from a bottle placed on the ground, in the center of the cages. The attractiveness was assessed 24 and 48 h after release, by counting the number of adults present on the abaxial surface of two leaves per plot, one from each plant belonging to the plot. After the final count, two leaves from each plot were sampled for leaf area measurements, in order to estimate the number of adultos/cm2, and counting the number of eggs on the abaxial surface. Genotypes 'Alicia AF-9354', 'Aline AF-9353' and 'Golden Delight' were the most attractive to adults of *B. tabaci* biotype B after 24 and 48 h of infestation. 'Sandy' and 'Daiane' showed low attractiveness to whitefly adults in both periods. 'Formosa' and 'Itapuã 301' express resistance of the non-preference type for oviposition. Genotype New Caravela proved to be the most susceptible.

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HOST PLANTS OF *BEMISIA TABACI* (GENN.), IN PANAMA

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Currently more than 500 *Bemisia tabaci* host plants have been reported, aggravating pest management, considering that it is a virus vector (Begomovirus), samples has been collected in agricultural crops, ornamental plants, bushes and shrubs during weekly intervals for a year in different locations, Tres Quebradas, La Espigadilla, Parita and Chitre; in Panama. The sampling performed weekly for a year period, considered the presence of 3º ninphal stage in *B. tabaci*, in order to be registered as a host. In each host considered plant, 15 leaves were collected, establishing as a collection pattern 5 leaves in each level (superior, medium and inferior). The identification of ninphal phase was performed through the ¨vasiform orifice¨ morphology. The results were analyzed by collection location and by host family plant. The results obtained indicate that the locations of La Espigadilla, Tres Quebradas, presented higher levels of infested plants with *B. tabaci*. The families that presented higher levels of infestation were: Sterculliaceae (23.5%), Solanaceaea (23%), Cucurbitaceae (15%), Malvaceae (6%) and Euphorbiaceae (27%), among others.

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NUTRITIONAL ADAPTATION OF *OEBALUS INSULARIS* STAL (HETEROPTERA: PENTATOMIDAE), *ECHINOCHLOA COLONA* LINK (POACEAE)

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The biological performance of *Oebalus insularis*, is associated to the nutritional quality of several food sources. For which this work determined the nutritional quality of the natural hosts *Oryza sativa, Echinochloa colona* and *Eclipta alba*), for the biological performance of *O. insularis*. The experiments were installed in laboratory conditions ($28+2^0$ temperature, relative humidity 80+5% and photophase of 8 hours). The following parameters were evaluated: a) Biological cycle duration; b) Longevity of the adults; c) Oviposition period; d) Eggs viability; e) Number of Eggs by posture; f) Number of postures by females. The experimental design was completely at random and the statistical analysis used was a ¨t¨ test at 5% probability level. In addition a fertility life table was developed considering: a) Liquid reproduction rate (Ro); b) Increase infinite rate (Rm); c) Increase finite rate (λ). The insects feeded with *E. alba*, only developed until second ninphal stage considering a natural inviable diet. The biological and reproductive parameters of *O. insularis*, obtained in *O. sativa* and *E. colona*, confirmed the nutritional efficiency and adaptation of the insect to *E. colona.* In addition, the parameters obtained in the fertility life table (Rm, Ro and λ), also confirm the nutritional efficiency of *E. colona*, in the biological performance of the pest.

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LABORATORY ASSESSMENT OF PEA-WHEAT PLANT ASSOCIATION COUPLED WITH POTENTIAL APHID INFESTATION ON *SITOBION AVENAE* BEHAVIOUR

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Semiochemicals are involved in multitrophic interactions, affecting the behaviours of both the herbivores and the beneficial insects. Several volatile molecules are emitted by infested plants but also from the herbivores. In most of the study, volatile organic compounds of herbivore-plant associations were assessed on the entomophagous beneficials. One way to promote biological control in crop pests is the management of fields by planting intercropping. In the proposed association, pea and wheat intercrops are proposed to reduce the pest abondance, including wheat aphids. In this work, a laboratory assessment of the association of pea and wheat was tested in behavioural test. Not only healthy but also aphid infested plants were tested in several combinations. Using a two way olfactometer, apterae and alate *Sitobion avenae* were observed when presenting different kinds of dual choices. Healthy plants were preferred by *S. avenae* to empty control. Also, the presence of conspecific on wheat proposed plant did not provide any more attraction to tested alate awheat aphids. The presence of *Acyrthosiphon pisum* infested pea induce a significant repulsive effect on *S. avenae*. These results were discussed to promote intercropping and aphid control in further field experiments including s the effect on beneficials in a push-pull approach by attracting the beneficial and repelling aphid pests.

Section 2

Xylem physiology and xylem-feeding insects

SOME METABOLIC ADAPTATIONS OF INSECTS THAT FEED ON XYLEM FLUID

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Xylem fluid is the most dilute and has the lowest chemical diversity of any plant tissue. The osmolality of xylem is typically 10 to 20 mM, and mainly consists of monomeric organic compounds (19 amino acids, 7 organic acids and 3 or 4 sugars), proteins (peroxidases, etc.) and inorganic ions. For Citrus and Vitis, the contribution of free amino acids in protein form is far less than free amino acids. The amino acid profile of xylem fluid is highly unbalanced. The amides, glutamine and asparagine, are the most abundant nitrogen form of transport in xylem fluid in most woody plants that we have investigated, and may account for over 75% or more of total free amino acids. Citrus xylem fluid is an exception where proline is a predominant amino acid. The chemistry of xylem fluid is highly variable within a plant species and is dependent on time of day, season of the year, soil fertility, and light levels. Xylem fluid is also under considerable tension, and ostensibly the extraction of fluid requires a considerable expenditure of energy. Thus, the chemical and physical properties of xylem fluid are in constant flux. Most of our leafhopper database is in reference to *Homalodisca vitripennis* Germar (formerly *Homalodisca coagulata* Say), but we have also examined *Homalodisca insolita* (Wlk) and *Cuerna costalis* F. Xylopagous leafhoppers are economically important since they transmit the bacterium, *Xylella fastidiosa*, which incites Pierce's disease, citrus variegated chlorosis and numerous scorch diseases in the Americas. The dilute nature of xylem fluid has led to several adaptations by xylophagous leafhoppers. They may feed at rates exceeding 100 times their dry body weight per day. *H. vitripennis* is highly polyphagous and may feed on hundreds of different plant species. Both the food source (xylem fluid) and insect excreta have been extensively analyzed. Host plant acceptance is based on gustatory sampling and is a function of the nutritional compliment of xylem fluid. Leafhopper abundance (host selection) is linked to the chemistry of xylem fluid. Our database indicates that glutamine is a phagostimulant for adult *H. vitripennis*. The N:C ratio of xylem fluid is higher than that any plant tissue and higher than that of pure protein. Leafhoppers ability to subsist on such dilute carbon diets is a result of assimilated efficiencies exceeding 99 % for organic compounds including amino acids, organic acids and sugars. The excretion of ammonia as a primary waste product confers maximum carbon assimilation efficiencies (95 to 99 %) and maximum caloric gain. By contrast, nitrogen retention is generally less than 60 % of that ingested. This pattern is opposite of that observed for aphids and other phloem feeders where 2 to 20 % dietary nitrogen and 70 % carbon is excreted. Feeding rates and the nutritional content of xylem fluid are generally highest during midday, whilst xylem tension is also at a maximum. The only experiment involving a water stress level that precluded feeding was when plants approached a condition of permanent wilt. The elucidation of chemical and physical plant properties in determining

leafhopper behavior and performance may have great utility in controlling *X. fastidiosa*-mediated diseases.

HOW DO SHARPSHOOTER LEAFHOPPERS FEED AND SURVIVE ON NUTRITIONALLY DEPAUPERATE XYLEM FLUID?

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Sharpshooters (Cicadellidae: Cicadellinae) are large, tropical and semi-tropical leafhoppers that are unique among all non-sessile hemipterans in ingesting primarily from xylem vessels. This presentation will summarize research on behavioral and physiological adaptations that permit sharpshooters to pump xylem sap under extreme tension, and survive on such a nutrient-poor food source. Sharpshooter stylet penetration is intracellular, and includes active uptake of fluid to precibarial chemosensilla to taste plant constituents both along the pathway to the xylem and inside xylem vessels, as demonstrated by electrical penetration graph monitoring of insect feeding, electromyography, and X-ray studies of cibarial muscle movements. Several xylem vessels can be punctured, tested, and abandoned before a suitable cell is found and accepted. The salivary sheath coats the point of puncture into a xylem vessel, forming a lining that chemically loosens and penetrates the cell wall. Thus, hardened saliva mechanically seals stylet tips into the xylem, preventing cavitation, as demonstrated via magnetic resonance imaging. Counterintuitively, sharpshooters apparently rely upon xylem tension to aid cibarial pumping, because such pumping becomes labored and eventually ceases when a host plant is not actively evapotranspiring. Cicadellines exhibit dietary mixing, i.e. feeding from numerous hosts to optimize consumption of certain xylem constituents. The majority of sharpshooters' time on plants is spent ingesting, resulting in large volumes of excreta (several times body weight in 24 hours). The highly efficient filter chambers of sharpshooters concentrate nearly all organic compounds in xylem fluid for absorption. Sharpshooters further conserve energy by being the only terrestrial organisms that excrete pure ammonia (ammonotelism). Finally, several species of endosymbiotic bacteria produce essential nutrients not found in xylem fluid. Thus, sharpshooter physiology is supremely adapted to subsist on xylem fluid.

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SUPPORT FOR THE SALIVATION-EGESTION HYPOTHESIS FOR *XYLELLA FASTIDIOSA* INOCULATION: SALIVARY GLUCANASE IS INJECTED INTO XYLEM DURING VECTOR FEEDING

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The salivation-egestion hypothesis for the inoculation mechanism of *Xylella fastidiosa* (Xf) proposes that saliva secreted into plants is taken up into the vector's precibarium. There, saliva loosens the Xf bacterial biofilm by enzymatically degrading the β-1, 4 glucans that form the chemical backbone of the exopolysaccharide matrix binding bacteria to the insect's cuticle. Bacteria-laden fluid is then egested into xylem vessels. The present study purified and tested enzymatic activities of all carbohydrases in the saliva of glassy-winged sharpshooter (GWSS), the primary economic vector of Xf. The most active salivary fraction was a type of cellulase, β-1,4 glucanase. Subsequently, over 2,000 pairs of GWSS salivary glands were dissected, extracted, and the glucanase purified, then used to produce a polyclonal antibody for immunohistochemical staining of GWSS salivary sheaths. Grapevine petioles were probed by sharpshooters recorded via electrical penetration graph (EPG), to determine when insects' stylets had reached xylem via production of an X wave. Grapevine tissues were excised, fixed, embedded in paraffin, sectioned, and examined using confocal scanning laser microscopy (CSLM). Results showed that red-stained glucanase-containing saliva co-localized (and therefore was secreted simultaneously) with sheath saliva for most of the probe. However, the narrowest branch(es) at the tip(s) of the salivary sheath were composed only of glucanase-containing saliva, which was also clearly injected into the terminal xylem cell. Glucanase-containing saliva formed a ring that lined the interior of the xylem vessel and penetrated into its cell wall. In addition, salivary lining of the xylem cell was found in adjoining sections, pulled over 200 µm downstream from the point of entry and connected to a salivary deposit in the middle of the xylem cell. Glucanase-containing saliva was also injected into non-xylem cells, where it accumulated in loose, flocculent masses. Results support the egestion-salivation hypothesis by showing that bacteria carried in glucanase saliva could be injected into xylem.

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SUPPORT FOR THE SALIVATION-EGESTION HYPOTHESIS FOR *XYLELLA FASTIDIOSA* INOCULATION: X-RAY STUDIES SUPPORTING THE EXISTENCE OF EGESTION

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The mechanism of inoculation of the Pierce's disease bacterium, *Xylella fastidiosa* (Xf), by vectors such as the glassy-winged sharpshooter (GWSS) is still unknown, despite nearly 70 years of study. Research in support of the salivation-egestion hypothesis for Xf inoculation is presented. Two important features of this hypothesis are: 1) uptake of saliva into the precibarium, causing attached Xf bacteria therein to loosen from the cuticle, followed by 2) expulsion (egestion) of saliva containing loosened bacteria into the xylem prior to ingestion. To directly observe actions of cibarial muscles controlling ingestion (uptake) and putative egestion of fluid from the precibarium, live, feeding GWSS were X-rayed and video-recorded in the Advanced Photon Source at the Argonne National Laboratory. Simultaneously, feeding of X-rayed sharpshooters also was recorded using AC-DC Electrical Penetration Graph (EPG) technology. Cibarial muscles were observed to be attached to two sets of tracheae inside the head. The tracheae moved at different times during feeding, clearly indicating muscle movement. Video indicated rhythmic pulsing of primarily dorsal tracheae was correlated with EPG waveform C2, whereas abrupt, non-rhythmic, dorsal tracheal movements were correlated with EPG waveform C1. Gentle fluttering of both sets of trachea was correlated with waveform B1. Results indicate that B1 is correlated with uptake of small amounts of fluid, presumably into the precibarium alone for tasting and possibly rinsing egestion of small amounts of fluid. C1 is correlated with rapid release of the cibarial diaphragm, probably powering discharging egestion from the cibarium. C2 is correlated with uptake of large amounts of fluid into the precibarium and cibarium, for ingestion (swallowing). Because the cibarial dilator muscles are the only means by which egestion could be performed, results provide indirect support for egestion.

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XYLEM NUTRIENT UTILIZATION AND THE LIFE HISTORY OF SHARPSHOOTER LEAFHOPPERS

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Our knowledge of how insects subsist on the dilute nutrients in xylem fluid has greatly increased, yet comparatively few studies have addressed the stages of life history when insects are most likely to be nutritionally constrained (immature development and egg production). Historically, the sharpshooter *Homalodisca vitripennis* Germar (Hemiptera:Cicadellidae) were difficult to rear on single host species; many of the common hosts for adult leafhoppers were insufficient for nymph development. In a series of experiments, we found suitable developmental hosts and identified the nutritional requirements for *H. vitripennis* development. Nutrients required for development varied greatly from nutrients preferred by adults. Whereas adults prefer to feed (high consumption rates and high adult abundance) on plants with high concentrations of amides in xylem fluid, nymph performance (survival, growth rates, developmental period) was greater on hosts with more 'balanced' amino acid profiles containing higher concentrations of 'essential amino acids' (those that most insects cannot synthesize). This disconnect between adult feeding preference and developmental requirements led us to test the 'Mother Knows Best' or 'Preference-Performance' hypothesis; do *H. vitripennis* preferentially oviposit on hosts where immatures will have high performance? In choice and no-choice tests using a wide variety of hosts, we found no relationship of oviposition preference with success in immature development. Xylem analyses for hosts utilized in the experiments confirmed the relationship between essential amino acids and nymph performance. Surprisingly, rates of ingested essential amino acids were also the best correlates to aspects of adult performance. We further tested the effects of xylem nutrients on both *H. vitripennis* behavior (host selection and consumption rates) and adult performance (survival, body weight gain and oviposition) in the field using a variety of *Prunus* germplasm at different times of the year. Nutrients mediated both behavior and performance, but different nutrients regulated behavior as compared to adult performance. Host selection was highly plastic as host preferences changed with season. However, glutamine was a consistent correlate to consumption rates and adult abundance throughout the experimental period. Rates of ingested essential amino acids were most tightly correlated to adult performance, but a sharp demarcation in allocations to life history parameters occurred seasonally with ingested nutrients being correlated to body mass gain early in the season and to fecundity rates six weeks later when *H. vitripennis* is at seasonal peaks. Further research is needed to elucidate if these changes in nutrient allocation are solely mediated by nutrient levels or by other abiotic or biotic factors. Insects that feed on xylem fluid ingest a nutritional source depauperate in organic nitrogen and carbon. Our experiments suggest that consideration of nitrogen form is equally important in order to understand nutrient utilization throughout the life cycle of sharpshooter leafhoppers.

APPLYING APHIDS AS BIOSENSORS FOR INVESTIGATING THE DYNAMIC DISTRIBUTION OF SYSTEMIC INSECTICIDES IN PLANTS

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Aphids are globally widespread pests on many agricultural and horticultural crops. Their feeding damage and ability to transmit plant viruses can cause significant loss in plant productivity and it could be considered to have major impact on yield. Effective aphid control requires systemic distribution of insecticides within plants in order to reach hidden individuals such as those on the leaf undersides. The investigation of active ingredient (AI) translocation within plants is therefore of major interest in insecticide research. Different types of bioassays allow the investigation of AI movement along the plant vascular system by recording aphid mortality on defined plant parts. The application could be either done to foliage or to roots. Data will show how the AI translocation either within the xylem or the phloem can be determined based on the biological response of feeding aphids. The relevance of plant physiology on resulting translocation patterns is also demonstrated. A special test set-up recording the honeydew production of infested aphids over time demonstrates how different AI kinetics affect translaminar mobility and root uptake. Field studies investigate the compound distribution in growing plants according to different application types, with the analytical AI quantification in the plant tissues revealing a very good agreement with derived information from aphid assays and therefore confirming the suitability of applying aphids as biosensors.

THE XYLEM AS A TARGET FOR HEMIPTERAN HERBIVORES

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The aim of this presentation is to describe the main physiological features of the xylem transport system, highlighting those aspects that affect the interaction with sap feeding hemipeterans. Plants, like animals, have a well developed and sophisticated transport system. Unlike the simple low pressure system in animals, the plant circulation system is comprised of a high pressure phloem integrated with a low and high pressure xylem. The anatomy, composition, biochemistry and biophysics of both xylem and phloem dominate interaction with those herbivores who would exploit the particular plant resource. The adaptations possessed by herbivores that access and feed from the xylem will be discussed in relation to the physiology of the xylem.

Structural features

Mature xylem consists of strong lignified/subersised tubes that form large open conduits for efficient transport of water and dissolved components. Such lignifications provide a mechanical barrier for any herbivore that exploits this conduit. The structure if the stylets appears more robust and the mechanism of feeding site location appears less sophisticated in exclusive xylem feeders compared to herbivores that mainly feed from the phloem.

Diet composition

Unlike the phloem individual xylem tubes are dead and have no direct membrane to regulate composition. Classically xylem is thought to transport sap of low concentration, with little or no organic content and particularly low in reduced nitrogen making it a poor diet for herbivores. Despite the direct association with a membrane, xylem composition can be tightly regulated. The casparian strip in the root endodermis necessitates the membrane passage of water and solutes entering the stele and so the xylem, thus providing a potential point of regulation for the plant. Once in the xylem, composition of sap moving up to the leaves is altered by active uptake into parenchyma and mesophyll cells. In addition the concentration of solute in xylem sap can vary by two orders of magnitude diurnally. However overall the dogma is that the xylem provides diet of low in carbon and nitrogen. Feeding studies indicate that xylem herbivores process large volumes of sap and are able to efficiently extract organic solutes.

Negative pressure

While flow in the sieve tubes is under the driven by high pressure generated osmotically, flow in the xylem during the day is driven by evaporation from open stomatal pores generating a negative tension in leaf cell wall water that is transpired by the cohesion of water into the xylem vessels and ultimately the root. This combination of cohesion and tension can generate huge negative pressure within the xylem fluid which can amount to minus 2-3 MPa.

Overcoming this negative pressure requires adaptations from any herbivore that can generate at least an equivalent negative pressure adding an additional energetic cost to xylem feeding during the day. The specific structure of the cibaral pump allows generation of sting 'suction' necessary for xylem feeding.
The negative pressure in the xylem represents and additional hurdle for a putative xylem feeding herbivore. Water under tension is under a meta stable state and xylem sap columns can snap cavitating the xylem and stopping transport. Cavitation is a serious problem for plants and can limit their distribution in xeric or freezing environments. It presents a serious problem for the xylem herbivore attempting to feed since introduction of feeding stylets would be expected to induce cavitation. However, current data suggests that many hemipteran are able to feed from xylem under tension, the mechanism by which they avoid cavitation have not yet been determined.

A variable food source?

At night or under low evaporative demand stomata shut and xylem pressures rise. During the night solutes are actively transported across the endodermis into the stele generating high osmotic pressure and therefore turgor. Under these conditions xylem pressures can reach 2 or 3 MPa positive pressure with sap containing many solutes. Under these conditions xylem sap represents a more favourable food source. However data on the diurnal feeding behaviour of xylem herbivores is not extensive. However it is clear that xylem feeding during the day under low pressure low concentration conditions is clearly undertaken.

Hemipteran osmoregulation

There are clearly hemiptera that have adapted to feed perhaps exclusively in the difficult environment of the xylem. However, detailed examination of the feeding behaviour of apparently exclusively phloem feeding hemipterans such as the aphids reveals that not only do they feed from the high pressure, low water potential environment of the sieve tube but also access the physically and chemically very different xylem. Indeed, the need for osmoregulation imposed by the high osmotic pressure of the phloem may require some rehydration from the xylem. The need for in apparently exclusively phloem feeding aphids explains why aphicides such as thiomethoxan (TMX) that are transported in the xylem can reduce aphid performance and open the possibility that novel control strategies could focus on herbivore osmoregulation mechanisms.

EVOLUTION OF XYLEM-FEEDING IN AUCHENORRHYNCHA, WITH EMPHASIS ON SHARPSHOOTER LEAFHOPPERS

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Auchenorrhyncha includes approximately 40,000 species of piercing-sucking "sheath feeding" herbivores, which typically seal their stylet tips into a vascular plant cell via a solid sheath made of saliva. The majority feed preferentially on phloem sap, while only cicadas (Cicadoidea), spittlebugs (Cercopoidea), and some leafhopper (Cicadellidae) lineages feed preferentially on xylem sap, notably the sharpshooters (Cicadellinae). Exceptionally, typhlocybine leafhoppers do not feed on vascular sap, but through a process called "cell rupture feeding", in which a sheath is not made and stylets move continuously or intermittently, lacerating cells, secreting watery saliva, and ingesting the resulting slurry of cell contents, usually from the mesophyll. Considering recent hypotheses of phylogenetic relationships among Auchenorrhyncha lineages, a transition to xylem sap specialization probably happened only once outside Membracoidea -- either in the ancestor of Cicadoidea + Cercopoidea or in the ancestor of Auchenorrhyncha. However, within Membracoidea this behavior was traditionally thought to be primitively retained by sharpshooters, which does not agree with current hypotheses of leafhopper phylogeny. A new combined phylogenetic analysis of morphological and 28S rDNA sequences (3,215 characters) of 139 terminal taxa, representing 87 of the 111 tribes of leafhoppers, will be presented and used to infer the number of origins of xylem specialization within Membracoidea. Unfortunately, information on feeding behavior is lacking for most leafhopper lineages, therefore assumptions on the behavior and correlated morphological characteristics will be discussed. Based on our results, it is expected that xylem-specialization originated at least twice during the evolution of this diverse group, which apparently had a phloem-specialist ancestor. Finally, another combined morphological and molecular (COI, COII, 16S rDNA, and Histone H3) phylogeny will be presented focusing on sharpshooters, the main vectors of *Xylella fastidiosa*. Changes in the higher classification of sharpshooters based on these phylogenetic results will be presented.

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THE ORGANIZATION OF THE LUMINAL SYSTEMA OF MEMBRANES IN THE MIDGUT OF THE SHARPSHOOTER *BUCEPHALOGONIA XANTHOPHIS* (BERG) (HEMIPTERA: CICADELLIDAE): A CHALLENGE OF PARADIGM?

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In the majority of insects, a peritrophic membrane (PM) envelopes the food bolus and compartmentalizes the midgut lumen and the digestive process. In Hemiptera, a PM is absent, but the enterocytes are not in direct contact with food, due to the existence of a luminal system of membranes known as perimicrovilar membranes (PMM), which considerably increases the surface area of the midgut epithelium. A typical PMM are found in some extant Heteroptera. On the other hand, in some species of aphids, a modified permicrovillar membranes (MPM) can be observed associated to the enterocyte microvilli. As a member of the Auchenorrhyncha suborder, the sharpshooter *B. xanthophis* was studied anatomically and ultrastructurally. For ultrastructural analysis by TEM, the midgut was fixed in modified Karnovsky solution in sodium cacodylate buffer, post fixed in 1% osmium tetroxide and embedded in Spurr resin. For lanthanum tracer study, 1% of lanthanum nitrate was added to sodium cacodylate buffer. The gut of *B. xanthophis* is composed of 3 basic regions: foregut, midgut and hindgut. The foregut is a simple slender esophagus. The midgut consists of a filter chamber (an association of the anterior and posterior midgut and Malpighian tubules), a conical and a tubular domain of the ventriculum. The hindgut is a long flattened tube and ends in the rectum. Ultrastructurally, all 3 epithelia of the filter chamber have their apical surface modified into microvilli, reduced cytoplasm and well developed basal membrane infoldings with mitochondria. The enterocytes have microvilli at the apex and a few basal membrane infoldings. Golgi and rough endoplasmic reticulum can be seen mostly around the nucleus. Secretion vesicles can be observed throughout the ventriculum. Associated with microvilli there is a perimicrovillar-like system of luminal membranes called flame-like luminal membranes (FLM), forming a closed compartment, according to lanthanum experimental results. The FLM originates from constrictions of the microvillar tips, which form membranes that projects into the lumen, keeping their association with the microvilli. Formerly, the PMM was considered as a structure present in the whole order Hemiptera, but the occurrence of a new kind of luminal system of membranes, which differs significantly from PMM and MPM, opens a new discussion concerning the actual evolutionary origin of these systems of membranes.

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DIFFERENT PLANT RESPONSE TO NYMPHS AND ADULTS OF SPITTLEBUGS (HEMIPTERA: CERCOPIDAE) – CASE OF PANICUM MAXIMUM CULTIVAR MASSAI

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Several species and genera of spittlebugs (Hemiptera: Cercopidae) are economic pests of forage grasses in tropical America. Damage caused by these insects can result in the complete loss of available forage, thereby reducing the carrying capacity of infested pastures. Host plant resistance is both a low-cost method of controlling insects and appropriate for low value crops per unit area, like pastures. Forage grasses germoplasms (*Brachiaria* spp. and *Panicum maximum*) are being screened for spittlebug resistance at Embrapa's Beef Cattle Research Center. Both nymphs and adults are xylem feeders. Nymphs feed mostly on superficial roots as well as at the base of the plant, while the adults feed mostly on the leaves. In the screening process two mechanisms of resistance have been considered: antibiosis to nymphs and, tolerance to adult damage. Although high levels of both resistance mechanisms are desirable in a given released cultivar, different plant responses have been recorded in this insect-plant relationship. The forage grass *Panicum maximum* cv. Massai has shown a high level of antibiosis to nymphs but a low level of tolerance to adult damage. As to the mechanism Antibiosis, lower nymph survival and prolonged nymphal period have been observed in the cultivar Massai, when compared to other *P. maximum* cultivars. Nymph survival percentages and duration of nymphal period of the pasture spittlebug *Notozulia entreriana* were 4% and 42,5 days in the cultivar Massai, contrasting to 32% and 29,6 days in the cultivar Tanzania; and 60% and 26,8 days in the cultivar Mombaça. As to the mechanism Tolerance, based on the reduction of dry matter production of plants compared under the same insect pressure (10 spittlebug adults/plant, during 10 days), a higher reduction was registered in the cultivar Massai. The percentage of dry matter reduction due to *N. entreriana* adult damage in the cultivar Massai was of 88,1%, while 58,8% and 38,6% reductions were observed in the cultivars Tanzania and Mombaça, respectively.

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Section 3

Other modes of piercing-sucking feeding

CELL RUPTURE FEEDING BY *EMPOASCA* AND *LYGUS* SPP. AND THE CAUSES OF THEIR PLANT DAMAGE

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Empoasca spp. leafhoppers (Cicadellidae: Typhlocybinae) and *Lygus* spp. bugs (Miridae) are the most-studied model species for the hemipteran feeding strategy formerly termed lacerate-and-flush feeding, recently re-named cell rupture feeding. Research using electrical penetration graph (EPG) monitoring, plant histology, and salivary biochemistry is summarized on the feeding biology of *E. fabae*, *E. kraemeri*, and *L. hesperus*. Cell rupture damage is caused by a dynamic interaction between insect stimuli and plant responses. *Empoasca* leafhoppers demonstrate a degree of behavioral plasticity not seen in other hemipterans. *E. fabae* and *E. kraemeri* can perform three tactics of cell rupture feeding, varying the mixture and durations of tactics on different host plants. In addition, each tactic triggers different local and systemic responses in the plant that lead to different, host-specific symptoms. Lacerate-and-sip, the most damaging tactic, consists of brief intracellular probes with secretion of watery saliva but very little or no sheath saliva. Stylets rapidly puncture multiple columns of stem phloem cells, causing much wounding laced with saliva. Phloem cell necrosis and abnormal meristematic development ultimately cause stunting and chorosis above the point of feeding. In the lacerate-and-flush tactic, stylets make longer intracellular probes to slowly puncture, salivate into, and drain mesophyll/parenchyma cells in the abaxial leaf surface, causing entry of air and tissue collapse. In the lance-and-ingest tactic, stylets puncture a phloem sieve element that briefly leaks phloem sap, which is ingested while stylets remain motionless. Saliva is released as stylets withdraw, leading to cellular hypertrophy of the adaxial leaf surface. The latter two tactics, together, cause leaf curling and necrosis. *Lygus* bugs are not so behaviorally plastic, and use only a fourth tactic, macerate-and-flush. *L. hesperus* makes brief probes to inject large amounts of watery saliva that contains highly active cell-wall degrading enzymes. After a period of non-probing quiescence, *L. hesperus* makes long probes to ingest macerated plant contents, leading to severe collapse and necrosis of plant tissues.

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CURRENT KNOWLEDGE ABOUT FEEDING BEHAVIOUR OF PLANTHOPPERS AND LEAFHOPPERS OF PHYTOSANITARY IMPORTANCE IN ARGENTINA (HEMIPTERA-AUCHENORRHYNCHA)

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Planthoppers and leafhoppers (Delphacidae and Cicadellidae) represent one of the most relevant groups within hemipteran owing to their importance for plant health. They have a role in the transmission and dispersal of virus, phytoplasms and spiroplasms which affects wild and cropped plants. To the present, the feeding behaviour of the following species was studied in Argentina: *Delphacodes kuscheli* Fennah (Delphacidae), the main vector of "Mal de Río Cuarto Virus" (MRCV) on corn; *Typhlocybella maidica* Catalano (Cicadellidae: Typhlocybinae) found at high densities on corn crops, and *Megamelus scutellaris* (Berg) (Delphacidae) and *Taosa (Cuernavaca) longula* Remes Lenicov (Dictyopharidae), planthoppers that feed and reproduce on the invasive aquatic weed, *Eichhornia crassipes* (Martius) Smols-Laubach (Pontederiaceae) and are considered potential biocontrol agents of this weed.
This contribution summarizes information about the experimental rearing methodology for these species, as well as the observations about feeding behaviour through histological examination of injured plant tissues using light microscopy, scanning and transmission electron microscopy. The feeding strategies of the species above are summarized as follows. The delphacids *D. kuscheli* and *M. scutellaris* and the dictyopharid *T. longula* are typical "salivary-sheath-feeders" ingesting mostly phloem sap. The typhlocybine, *T. maidica* is a "cell rupture feeder" consuming the mesophyll cell contents but not making true salivary sheaths. However, tenuous salivary sheaths in relation to vascular tissues could be indicates the ingestion of phloem sap by *T. maidica*. The alteration of the normal structure of chloroplasts and the partial or total occlusion of the cellular lumen of vascular tissues are the main damages produced during feeding mechanism of these species.

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BEYOND VASCULAR TISSUE: TACTICS FOR FEEDING ON PARENCHYMA, EPIDERMIS, AND REPRODUCTIVE STRUCTURES OF PLANTS

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Electrical penetration graph techniques have been extensively used to analyze probing to phloem sieve tubes and xylem vessels, but only recently has this procedure been adapted for use with Heteroptera and other piercing-sucking feeders that exploit ground, dermal, and meristematic tissues. Damage to these tissues may result in chlorotic lesions, stunting or necrosis of new growth, fruit deformities, cone abortion, seed losses, and pathogen transmission. Ingestion may occur from a variety of cell types, including spongy and palisade mesophyll, phloem parenchyma, root storage parenchyma, pericarp, embryo, endosperm, and nucellar tissue. As we increase our understanding of these diverse feeding behaviors, the traditional distinctions between “sheath feeders” and “lacerate (or macerate)-and-flush” feeders may be blurred, and guild concepts of xylem, phloem, and mesophyll feeding modes need to be expanded. Emergence of pentatomids as serious pests of soybean and genetically engineered cotton worldwide has spurred greater interest in EPG analysis of probing and plant damage by pentatomomorphan true bugs. Research with green vegetable bugs, *Nezara viridula* (L.) (Pentatomidae) and leaffooted bugs, *Leptoglossus phyllopus* (L.) (Coreidae), using an AC-DC four-channel universal EPG monitor, has elucidated distinct waveform types associated with stink bug pod and stem feeding. Coreid waveform patterns on legume pods are more varied than those of the pentatomids. Measurements of probe duration and frequency in leaffooted bugs have revealed differences among developmental stages; adults probe more frequently than large nymphs (3^{rd} – 5^{th} instar), locate the ingestion target tissue faster, and probe for a longer total period of time; time until first probe is also significantly shorter for adults than large nymphs. Correlation of waveform patterns with feeding behaviors is currently planned or in progress in other labs for a number of heteropteran pest species, including squash bug, *Anasa tristis* (De Geer); redbanded stink bug, *Piezodorus guildinii* (Westwood), brown stink bug, *Euschistus servus* (Say), and Southern chinch bug, *Blissus insularis* Barber.

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HETEROPTERAN SYMBIONTS: RECENT ADVANCES AND PERSPECTIVES

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Symbiotic associations within insects and microorganisms are of great importance. In Heteroptera, symbionts can be found in the insect´s lumen or within the gastric caeca. By now, caeca-associated symbionts are shown to be related with some insects of the families Plataspidae, Pentatomidae, Alydidae, Phyrrochoridae, Acanthosomatidae, Scutelleridae, Coreidae, and Parastrachiidae. Insects of the family Pentatomidae are pests of economically important crops worldwide. Due to advances on molecular biology, the relationship between pentatomid stink bugs and symbionts are been object of intensive studies. Caeca-associated symbionts are vertically transmitted through generations in an orally maner. First instar nymph's acquire the symbionts probing on the chorion surface of the eggs, smeared by the females, after it laid the eggs. Recently, it was shown that pentatomid insects carry a dominant symbiont in the gastric caeca. The symbionts are polyphyletic, divided within at least three groups of bacteria (i.e., plant pathogens (*Pantoea* sp.), facultative and obligatory intracellular symbionts). Scanning electron microscopy showed that the surface sterilization of egg masses may eliminate the microorganisms found on the surface of the chorion, but also it can remove some structures of the egg's surface. Surface sterilization of egg masses and high temperatures (30ºC) eliminated caeca-associated symbionts of *Nezara viridula*, but did not affect nymph's development. However, females originated from sterilized egg masses, when kept at low temperature (20ºC), never lay eggs. Interestingly, for the species *Acrosternum hilare* and *Murgantia histrionica* it was showed that high temperature affects the symbiotic relationship, with concomitant reduction in insect fitness. On the other hand, nymphs of the species *A. hilare* and *Pellaea stictica* from surface sterilized eggs showed high mortality. Only a few nymphs turn to female, but never laid eggs. According to the results, the degree of mutualism of the association is variable within the pentatomid stink bugs. Several factors, such as environment and/or age of the association can explain the different levels of dependence. Future directions include the need to better understand the biology of insect-symbiont associations, as well as the consequences of local climate changes for the dynamics of these interactions.

QUANTITATIVE ANALYSIS OF FEEDING BEHAVIOR OF SOUTHERN CHINCH BUG, *BLISSUS INSULARIS* BARBER (HEMIPTERA: BLISSIDAE), ON RESISTANT AND SUSCEPTIBLE ST. AUGUSTINEGRASSES

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St. Augustinegrass is the most widely planted turfgrass in Florida and other Gulf coast States in the United States. The southern chinch bug, *Blissus insularis* Barber, is the most serious insect pest of St. Augustinegrass. Host plant resistance has been one of the most successful pest management methods for this insect. 'Floratam', a polyploid variety of St. Augustinegrass with resistance to southern chinch bug has long been but few populations of southern chinch bugs have developed resistance to this variety. Although significant progress has been made in identifying new sources of southern chinch bug resistance in St. Augustinegrass lines, such as the polyploid FX-10 and the diploid NUF-76, the mechanisms of resistance in these lines are unknown. Previous studies reported high levels of antixenosis in both lines and possible antibiosis in NUF-76. Understanding the feeding behavior of southern chinch bugs on the resistant FX-10 and NUF-76 is important to elucidate the mechanisms of resistance. For the first time, the electrical penetration graph (EPG) technique was used to quantify southern chinch bug feeding behavior on resistant and susceptible St. Augustinegrass lines. Southern chinch bugs made more frequent probes, produced longer-duration waveform events for pathway-related behaviors (searching for an ingestion site) and spent less time in ingestion-related waveforms on FX-10 and NUF-76, compared to the susceptible Floratam and Palmetto. Relatively more stylet probes per insect on FX-10 and NUF-76 than on Floratam and Palmetto suggest the presence of stylet penetration impediments around the vascular bundle in resistant varieties. In addition, the short duration of presumed phloem sap ingestion on FX-10 and NUF-76 suggests the possible presence of resistance factors in phloem sap or blockage of sieve elements.

WAVEFORM LIBRARY FOR CHINCH BUGS (HETEROPTERA: LYGAEIDAE): CHARACTERIZATION OF EPG WAVEFORMS AT MULTIPLE INPUT IMPEDANCES

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Chinch bugs, *Blissus* spp. (Heteroptera: Blissidae), are among the most serious pests of grasses in North America. Southern chinch bug, *B. insularis* Barber, is the most important pest on St. Augustine- grass, the primary lawn grass in Florida. Western chinch bug, *B. occiduus* Barber, is a key pest on wheat and buffalograss, a common lawn grass in the western USA. Resistant grass and wheat cultivars have reduced chinch bug damage below economic thresholds for many years; however, resistance has often failed recently, and research efforts are underway to develop new grass cultivars resistant to these pests. Past studies have shown that chinch bugs are salivary sheath feeders that ingest primarily from phloem sieve elements. To support concurrent studies using electrical penetration graph (EPG) to compare chinch bug feeding among resistant and susceptible grass accessions, EPG waveforms were recorded for the first time for *B. insularis* and *B. occiduus* using a 4-channel version of the Backus and Bennett AC-DC EPG monitor. Waveforms were characterized for both AC and DC applied signals, using input impedances of 10^6, 10^7, 10^8, 10^9 and 10^{13} ("emf only") Ohms. A matrix of waveform appearances (a "waveform library") at both types of applied signal and all input impedances is provided. Electrical origin (R, emf or both components of the output signal), voltage levels, repetition rates, and waveform appearances are provided for waveform types described. An input impedance of 10^7 Ohms, using either AC or DC applied signal, appears to provide the best balance of R and emf components, for future studies. Although histological and biochemical correlations were not performed, tentative biological meanings are assigned based on similarity to many other species' waveforms. Three putative pathway waveforms, two putative ingestion waveforms, and a hypothesized X-wave are characterized.

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PHLOEM OR XYLEM FEEDING? USE OF VEGETATIVE AND REPRODUCTIVE PLANT PARTS AND TISSUES BY TWO LARGE NEOTROPICAL COREIDS

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Due to physiological and morphological constraints, large-sized hemipterans are predicted to more frequently use xylem than small ones. In addition, hemipterans with high mobility (e.g., true bugs) are able to use a wide range of plant parts, leading to an increase in diet range. The large neotropical coreids *Holymenia clavigera* Herbst and *Anisoscelis foliacea marginella* Dallas (Anisoscelini) were observed foraging on several plant parts of their hosts – Passifloraceae – and were thus examined with respect to feeding preferences and host use under laboratory and field conditions, as just a few studies have been devoted to this topic in tropical heteropterans. Histological sections of *Passiflora suberosa* L. were performed on feeding sites, on plant pieces having the penetrated stylet in situ. Both nymphs and adults of *H. clavigera* and *A. foliacea marginella* fed on all *P. suberosa* parts. First instar nymphs preferred the terminal buds, shifting to immature fruits after molting. When using leaves, the stylets reached the xylem, in almost all situations, followed by a low use of phloem. Nymphs and adults of both species consumed several fruit parts, including the seeds. When feeding upon the latter, endosperm and embryo were used for feeding. These findings agree with the general statement that large-sized hemipterans tend to use xylem. As seeds are the main resource used by these coreids, xylem feeding is probably related to water acquisition. Overall, this study highlights the food mixing condition of *H. clavigera* and *A. foliacea marginella*, as well as the importance of fruits for their nutrition.

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FEEDING BEHAVIOR AND SUPERFICIAL DAMAGE TO SOYBEAN SEED BY *EDESSA MEDITABUNDA* (F.) AND *EUSCHISTUS HEROS* (F.) (HETEROPTERA: PENTATOMIDAE) IN THE GREENHOUSE

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Greenhouse studies were conducted to evaluate the feeding behavior and superficial damage to soybean seed by two pentatomid species, *Edessa meditabunda* (F.), and the Neotropical brown stink bug, *Euschistus heros* (F.). In the greenhouse, soybean plants (cv. BRS 282), at R6 stage of development were used, with pods and stem isolated with small plastic cages (6.0 cm diameter). Thirty couples of each species were used, each couple placed individually in each cage for 48 hours. Two daily observations (9 AM, and 3 PM) were taken, and the number of bugs (feeding or not) on the different plant parts was recorded. At maturity (R8 stage), seeds were harvested, imbibed in tetrazolium solution (2,3,5-triphenyl tetrazolium chloride), and placed in a germination chamber (25 ± 1^{o}C) for 24h. Seeds were photographed into 20 x 20 mm square for measurement of the superficial damaged area using Photop software. The number of *E. meditabunda* on the plant structures was similar at 9 AM (mean number of 28.0 bugs), and 3 PM (24.3). Adults significantly ($P<$ 0.01) preferred soybean stems (19.7 bugs) than pods (2.7). The majority (52.6%) of adults fed upside down. The number of *E. heros* on the plant structures was 13.7 bugs at 9 AM and 17.7 bugs at 3 PM, and were equally recorded on stems (7.3 bugs) and on pods (6.9); however, most insects (12.3 bugs) spent time on the cage net. The superficial damage to seeds (area of the cotyledon marked with tetrazolium solution) was significantly ($P<$ 0.01) greater for *E. meditabunda* (22.89 mm^2) compared to *E. heros* (12.47 mm^2).

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DISPELLING THE RASPER MYTH AND INVESTIGATING HOW VIRUS INFECTION CHANGES THRIPS FEEDING BEHAVIOR

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Long ago people discovered extraordinarily tiny yellow to brown insects (thrips!) that seemed to be responsible for causing unsightly areas of silvery damage flecked with dark spots on the leaf surface. Indeed, thrips feeding is a serious problem for a multitude of food, fruit and fiber crops. How could such a small insect inflict such large areas of damage? Several researchers proposed that thrips must utilize a feeding strategy somewhere between sucking and chewing. A simple statement made in 1915 that thrips exhibit a "sort of rasping movement" to break open cells and then suck up the contents, led to classification of thrips as raspers or rasping-sucking insects. This classification can be found in numerous entomology text books and web based fact sheets despite many reports showing that thysanopteran mouthparts do not have any structures for rasping. Thrips have asymmetrical stylets that clearly serve in piercing cells and sucking out plant fluids, composed of a single mandibular peg and a pair of maxillary stylets that form a feeding tube. Video recordings and electrical penetration graph recordings of feeding confirm that thrips utilize a "piercing-sucking" feeding strategy. These recordings show that the very visible silvery damage caused by thrips feeding arises from the emptying of a large number of individual cells. I have shown that thrips engage in three basic types of probing behaviors: non-ingestion probes, short ingestion probes, and long ingestion probes. During non-ingestion probes thrips do not actively ingest plant sap but are likely sampling cell contents to determine suitability of the food source. In short ingestion probes thrips suck out individual cells, and during long ingestion probes thrips engage in sustained ingestion, from an unknown food source, possibly the xylem. Even more important than the direct feeding damage thrips cause is the damage inflicted by the viruses they transmit. Several species serve as vectors of *Tomato spotted wilt virus* (TSWV) type member of the tospoviruses (the only plant-infecting genus in the *Bunyaviridae*), and a devastating plant virus that infects over 1000 species of plants. I have recently shown that infection with TSWV alters the sexually dimorphic feeding behavior of its thrips vector, *Frankliniella occidentalis* (Pergande) in a sexually dependent manner. Male thrips infected with TSWV fed more than uninfected males, with the frequency of all feeding behaviors increasing by up to 3 fold, thus increasing the probability of virus inoculation. Importantly, infected males made almost 3 times more non-ingestion probes compared to uninfected males. Non-ingestion probes leave cells largely undamaged and able to support virus replication and movement. Furthermore we have shown that thrips salivate immediately before and during these probes indicating that this behavior is most likely to establish virus infection. Although several plant viruses infect their insect vectors, this has been the first report that vector infection by a plant virus alters feeding behavior.

JUMPING PLANT-LICE AND HOST PLANTS INTERACTIONS, DAMAGES ON CULTIVATED PLANTS AND FOREST TIMBERS IN CAMEROON

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Jumping plant-lice or psylloids form a moderate-size group of Hemiptera Sternorrhyncha. They feed on plant-sap, usually from the phloem, and are generally highly host specific. Psylloids are usually associated with dicotyledons, and related species often develop on related host taxa. They can be harmful to their angiosperm hosts in removing large quantities of plant-sap, and in producing honey dew which soils leaves and fruits, and attracts sooty moulds, or by transmitting diseases. Recent investigations on psyllid biodiversity in Cameroon enable us to described new species from this country. The biology of psyllids was also studied during the last decade in Cameroon. The present work described the interactions between twenty psyllids species and their hosts in Cameroon. The damages that each psyllid species caused on his host have been raised. The visible damages, caused during larval stages, were mainly the formation of the galls, the enrolment of leaves, the burns and necroses on the plant tissue, the apparition of the wounds following to the egg insertion mode into the plant tissue. In some cases, the pit galls persist after the last moult of the insect. The damages caused by psyllid to the plant tissue are irreversible. *Phytolyma fusca* caused closed galls on leaves and buds of *Milicia excelsa*; larvae dig galleries that assure the communication between different galls. These galls persisted during larval stages and explode during the emergence of adults. Leaves and buds thus attacked dried and degenerated. *Pseudophacopteron pusilum*, psyllid of *Dacryodes edulis*; *P. fuscovenosum*, psyllid of *Deimbolia* sp.; *P. nothospondiadis*, psyllid of *Nothospondias letestui*; *Trioza* sp., psyllid of *Schefflera barteri* provoked closed and isolated galls on leaves of their respective host. These galls explode at the end of the larval development; the buds and leaves does not dry after the emergence of adults. *Trioza erytreae*, citrus psyllid; *Trioza* sp., psyllid of *Stephania abyssinica*; *Trioza* sp., psyllid of *Drypetes leonensis* caused pit galls or crypts on leaves of their respective host. These galls are opened at the lower face of the leaves. Heavy infestation spoiled the appearance of young trees of citrus. *Colophorina* sp., psyllid of *Deutarium macrocarpum*; *Afrotrioza* sp., psyllid of *Bersama* sp.; *Symtomosa* sp., psyllid of *Homalium letestui*; *Pseudophacopteron cuniculus*, psyllid of *Bligia unijugata*; *Diclidophlebia xuani*, psyllid of *Ricinodendron heudelotii* provoked galls resulting from the enrolment of young leaves that hold larvae and adults. Other psyllids species produced large quantities of honey dew responsible for the burns of the leaf tissue: Psyllinae sp., psyllid of *Pitosporum viridiflor*um; *Triozamia* sp., psyllid of *Antiaris africana*, *Paurocephala* sp., psyllid of *Dombeya ledermannii*; *Heteropsylla cubana*, psyllid of *Leucena glauca* and *Trioza messii*, psyllid of *Caloncoba welwitchii*. The egg laying insertion mode of *Mesohomotoma tesmanni* provokes some wounds in the leaves tissue of cocoa. *Pseudoeriopsylla* sp. attacks roots of *Ficus thonningii*.

Most of the hosts are cultivated plants or forest timbers with economical value and pharmaceutical used in Cameroon.

THOSE CRAZY COREIDS! WHAT EPG TELLS US ABOUT SQUASH BUG FEEDING BEHAVIOR

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Host acceptance and activities that are part of plant feeding are fairly well understood for aphids, whiteflies, many leafhoppers and other taxa within the Hemiptera, but true bugs are not so well studied. Part of this is because unlike homopteran insects, most heteropterans were considered to be nuisance species and few were known to transmit phytopathogenic agents. Recently, this assessment has changed and with it has followed detailed histological studies of plant-feeding Heteroptera followed by electrical penetration graph analyses of two taxa in particular, the Miridae and the Blissidae. Another family has a few representative species that have been studied by EPG: the Coreidae. This group of insects is so interesting because, like the cicadellids, there appears to be a wide range of feeding strategies exhibited by this group based upon histological examination of salivary sheaths. So few coreids have been examined using EPG that generalizations about the group may be premature. However, the EPG recording of one coreid, *Anasa tristis*, the common squash bug, sheds some light on this interesting leaf and fruit feeder. Several distinct waveforms have been described including those associated with stylet pathway and ingestion. On a preferred host, squash, this insect probes repeatedly before initiating sustained ingestion. On less preferred hosts, squash bugs delay probing but will eventually ingest from plant tissues. Histological examination of sheath termination points suggest that ingestion is primarily from xylem, but phloem ingestion cannot be dismissed. This insect transmits a phloem-inhabiting bacterium to cucurbits suggesting that it accesses phloem tissues during feeding. However, no specific phloem-associated EPG waveforms have been uncovered. It may possible that *A. tristis* accesses phloem sugars via an osmotic pump strategy first proposed for the Coreidae by Miles.

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Section 4

Plant physiological and molecular responses to hemipteran feeding

OXYLIPINS IN PLANT DEFENSES AGAINST APHIDS

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Oxylipins represent a large, diverse group of compounds generated through oxidation of polyunsaturated fatty acids, and a variety of plant oxylipins have been implicated in signaling and defense. The objective of this project is to investigate the role of oxylipins in plant defenses against aphids using the interaction between tomato (*Solanum lycopersicum*) and the potato aphid (*Macrosiphum euphorbiae*) as a model. Jasmonic acid, which is among the most extensively-characterized plant oxylipins, activates induced defenses against caterpillars and other chewing insects; however, we have found that inhibiting jasmonic acid synthesis does not significantly impair plant defenses against aphids. Surprisingly, reducing production of C6 volatiles through antisense suppression of a 13-lipoxygenase (LOXC) in tomato also did not influence aphid population growth. Aphid infestation induced production of 9-lipoxygenase products in tomato foliage, suggesting that 9-LOXs may play a role in induced defenses against aphids. Aphids also upregulated expression of an α-dioxygenase involved in oxylipin synthesis, α-DOX1; furthermore, virus-induced gene silencing of α-DOX1 enhanced aphid population growth, indicating that this gene plays a role in limiting aphid infestations. These results provide novel insights into the contribution of oxylipins to plant defenses against sap-feeding insects.

TOMATOES THAT REPEL WHITEFLIES

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The world-wide spread of pest insects has locally led to large economic losses in crop production. We study the interaction between tomato (*Solanum lycopersicum*) and whiteflies (*Bemisia tabaci*) with the aim to increase the plant's repellence through modification of natural volatile emission and related toxins, specifically in glandular trichomes (leaf- and stem hairs). Plant semiochemicals play an important role for insects in locating a host plant and we investigated which volatile cues might be in involved in attraction/repellence of the phloem-feeding pest-insect *B. tabaci*. We first tested the preference of *B.tabaci* for a collection of wild tomato accessions and introgression lines, and determined the volatiles in the plants's headspace. Correlation analysis revealed that several terpenes were putatively unattractive for whiteflies. Terpenes are plant-produced compounds with a wide variety of functions, but well-known for their role in plant insect interactions and for their role in plant defence. Several of these candidate compounds conferred repellence to otherwise attractive tomato plants when applied to the plant. In particular the tomato-produced sesquiterpenes 7-epizingiberene and *R*-curcumene were shown to be active as semiochemicals to *B. tabaci* adults. However, the stereo-isomers zingiberene and *S*-curcumene, isolated from ginger oil, did not evoke a repellence response. Glandular trichomes are specialized plant tissues, well equipped for the production, storage and emission of terpenes. In order to clone the relevant sesquiterpene synthases we analyzed the transcriptomes of trichomes of relevant tomato lines by means of high-throughput sequencing (GS Titanium, 454 Life Sciences, USA) of ESTs. This allowed us to identify not only terpene synthases but also the genes involved in precursor biosynthesis. Moreover, to control/modify the production of relevant sesquiterpenes, we cloned several glandular trichome-specific promoters in order to drive these precursor genes and terpene synthases in transgenic tomato lines. We have now obtained several stable transgenic lines over-expressing different precursor genes in glandular trichomes. These lines have higher levels of precursors for terpene biosynthesis and will be crossed with transgenic lines that overexpress specific terpene synthases. Preliminary data show that this approach can indeed lead to the production of volatiles in cultivated tomatoes that act as repellents to whiteflies.

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HOST-PLANT MEDIATED INTERACTIONS BETWEEN BELOWGROUND EFFECTS OF ORGANIC AND CONVENTIONAL FARMING ON THE ABOVEGROUND PLANT-HERBIVORE RELATIONSHIPS

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It is known that belowground biogeochemical systems can influence the aboveground system. Agricultural practices involving fertilisers, pesticides, water, biological organisms, all have an important effect on the belowground ecosystems and these in turn affect the plant, herbivore, natural enemy complex aboveground. On the other hand, it is known that in several insect species, previous infestation with conspecifics has either a positive or negative influence on insect acceptance and/or colonisation of its plant host. Previous work by the author has shown that *Myzus persicae* adults are more attracted to conspecific-infested potato plants, but spend significantly less time probing and lay fewer eggs than on uninfested plants. In spite of this definite host preference exhibited by the insects, the effect on performance (in terms of egg hatch, percentage nymphal survival, percentage adult survival and fecundity of emerging adults) was found to be only minimally better on uninfested plants. Studying the effect of belowground factors on this insect host-plant relationship, using organic (compost) and conventional inputs (synthetic NPK fertilisers) showed that organic inputs were less favourable for the pest in general, and caused a behavioural change in the insect (the preference for uninfested plants appeared to be lost and insects colonised conspecific-infested and uninfested plants in equal measure). Furthermore, preventing the release of biologically-sourced soil volatiles by sterilising the soil also had a strong effect on the above ground insect -host plant interaction, with a greater preference being exhibited by *M.persicae* adults for conspecific infested host plants than uninfested ones. These results have implications for crop protection in organic and conventional agriculture.

MOLECULAR RESPONSE OF DIPLOID WHEAT TO GRAIN APHIDS

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The grain aphid (*Sitobion avenae*) is a major insect pest of cereal crops, acting as a virus vector as well as causing direct plant damage. The molecular responses of a commercial wheat variety (Claire) to infestation by *S. avenae* were investigated in order to identify defence response genes/products for a directed strategy for wheat breeding programmes (Ferry et al., 2011). The results showed that Claire, one of the most commonly grown varieties in the UK, lacks a specific insect defence response, although general response genes were shown to be present. The present study was carried out to investigate the potential of a diploid wheat line (ACC20 PGR#1755) exhibiting resistance to *S. avenae* to serve as a source of resistance genes, using a proteomic-based approach. Approximately 200 protein spots were reproducibly detected in leaf extracts from both the resistant and a susceptible (ACC5 PGR#1735) line using 2-dimensional gel electrophoresis and Progenesis SameSpots software. Twenty-four spots were significantly up-regulated (> two-fold) in the resistant line after 24h of aphid feeding (13 and 11 involved in local and systemic response, respectively). In the susceptible control, sixteen protein spots were significantly up-regulated after 24h aphid feeding, with 12 being involved in the local response and 4 involved in the systemic response. After 8 days, there was a further increase in the number of differentially expressed proteins in the resistant variety (43 spots were significantly up-regulated; 37 locally and 6 systemically), whereas in the susceptible line the number of differentially expressed protein spots decreased to 12 (8 locally and 4 systemically). Approximately 50% of all differentially expressed protein spots were identified by peptide mass fingerprinting, revealing that the majority of proteins up-regulated by aphid infestation were involved in metabolic processes (including photosynthesis) and transcriptional/translational regulation. However, in the resistant line several antioxidant and stress response proteins were identified as well as those involved in DNA synthesis/replication/repair. Interestingly, no antioxidant or stress response proteins were up-regulated in the susceptible line in response to aphid feeding. These data suggest that the antioxidant, stress response and DNA synthesis/replication/repair proteins play a role in conferring resistance to *S. avenae* in the diploid line studied.

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FUNCTIONAL CHARACTERIZATION OF EFFECTOR PROTEINS THAT MODULATE PLANT-INSECT INTERACTIONS

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Hemipteran insects use their piercing mouthparts to feed on various plant tissues and transmit a number of plant diseases, including viruses and bacterial pathogens such as phytoplasmas. In my lab we have generated evidence that the Pathogen-Associated Molecular Pattern (PAMP) Triggered Immunity (PTI) pathway plays a role in plant defence response to aphid attack and that a specific aphid effector suppresses PTI. Furthermore, silencing of a number of candidate effector genes in aphids by plant-mediated RNAi reduces the performance of aphids on plants, while expression of these effectors in plants increases progeny production. Only effectors from the aphid species tested showed the progeny-increase phenotype but not the effector homologs from another species. Phytoplasmas have effectors that modulate plant-insect interactions as well. Leafhoppers that vector aster yellows phytoplasma (AYP) produce more progeny on AYP-infected plants. We found that one phytoplasma effector destabilizes specific plant transcription factors that positively regulate genes involved in jasmonate production leading to an increase in leafhopper progeny. Phytoplasma effectors modulate plant development inducing flowers that become leafy, increased production of stems (witch's broom phenotype) and changes in leaf shapes that are also observed in symptomatic AYP-infected plants. Investigations of how these effectors may affect plant-insect interactions are ongoing. In conclusion, we generated evidence that various effectors can modulate plant-insects interactions providing new avenues for research that can lead to better control of hemipteran insect pests and the diseases they transmit.

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DISTINCT AND COMMON REQUIREMENTS FOR *MI-1*-MEDIATED RESISTANCE TO APHIDS AND ROOT-KNOT NEMATODES

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The tomato *Mi-1* gene, encodes a nucleotide binding leucine-rich repeat (NB-LRR) protein, is an atypical resistance gene as it confers resistance to wide ranging groups of pests. How *Mi-1* triggers plant defense to pests as diverse as root-knot nematodes (RKN) and phloem feeders like potato aphids, whiteflies and psyllids is poorly understood. We have used high-throughput virus-induced gene silencing (VIGS) and microarray approaches to identify genes in the *Mi-1* signalling pathway. The VIGS screen utilized a *Nicotiana benthamiana* cDNA library in tobacco rattle virus and assessed the suppression of a pest-independent hypersensitive response triggered by a constitutive active form of Mi-1, Mi-DS4. This screen identified several genes that suppressed *MiDS4* cell death. Among these is Somatic Embryogenesis Receptor Kinase 1 (*SERK1)* a LRR transmembrane receptor kinase. Interestingly, silencing *SERK1* in tomato attenuated *Mi-1*-mediated resistance to aphids but not to RKN demonstrating for the first time a distinct requirement for *Mi-1* resistance to these two pests. Using microarray analysis we found the paralogous tomato *WRKY* genes, *SlWRKY72a* and *-b* to be transcriptionally up-regulated during *Mi-1* resistance to RKN. Silencing these two genes in tomato resulted in a reduction of *Mi-1*-mediated resistance as well as basal defense against RKN and aphids. In addition, using Arabidopsis T-DNA insertion mutants we found their Arabidopsis ortholog, *AtWRKY72*, also to be required for basal defense to RKN as well as to the oomycete *Hyaloperonospora arabidopsidis*. *At*WRKY72 target genes, identified by microarray analysis, appear largely to be non-responsive to salicylic acid (SA) defense hormone analogs indicating that *At*WRKY72 utilizes SA-independent defense mechanisms.

WHITEFLY PREFERENCE IN TOMATO

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Silverleaf whitefly (*Bemisia tabaci* Genn.) is one of the most important pest in tomato. *Bemisia tabaci* causes direct damage through feeding and indirect damage through virus transmission. Whitefly control is mainly based on pesticide applications, but a promising alternative is the use of whitefly-resistant plants. Resistance has been found in several accessions of tomato wild relatives, such as *Solanum peruvianum*, *S. habrochaites*, *S. cheesmanii*, *S. pennellii* and *S. pimpinellifolium*. The aim of this study was to evaluate preference of *B. tabaci* for a selected set of tomato genotypes and to identify the metabolites underling the preference behaviour. A dual choice assay was performed to test the preference. The variables analysed were adult settlement and the preference for oviposition. The metabolic profiling was carried out using gas chromatography-mass spectrometry, and the compounds were extracted from the leaf with dichloromethane. Non-preference for both adult settlement and oviposition was found in two accessions of *S. habrochaites* assessed and in the line FCN 93-6-2. More than 149 different secondary metabolites were detected in the tomato leaf samples. From those a few were found in higher/lower concentration in the group of plants that showed non-preference, indicating that these compounds may play a role in the choosing behaviour. The lines were genotyped using a recently developed Infinium array that contained 6000 SNP markers. From the genotypic data we could infer that the region providing the non-preference may be located on the chromosomes 6 and/or 11.

ASSESSMENT OF APHID SALIVA ROLE AS PLANT DEFENCE ELICITATION BY MULTIPLE APPROACHES

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Interactions between plants and insects are numerous, complex and varied. Indeed, feeding behaviors of many insects are directly linked with defensive compounds in host plants. Until now, most studies were based on plant defense mechanisms associated with chewing insects. According to a theory, each species of herbivore insects produces its own particular molecular signature, especially concerning saliva. The role of insect saliva is crucial concerning establishment of defense mechanisms in plants because composition of saliva allows plants to recognize insects. Up to now, only few studies focused on the identification of elicitors in aphid saliva and the determination of elicitation activity. For this study, we used several approaches on tobacco and *Arabidopsis thaliana* whole plants and cell cultures to assess the potential elicitation activity of *Myzus persicae* saliva. After evaluating the early defensive responses of the plants using cell cultures by following the pH evolution and oxidative burst, protein pattern changes in the plants were assessed by developing two dimensions differential in gel electrophoresis (2D-Dige) coupled with mass spectrometry for protein identification. Both models, whole plants and cell cultures were shown to be considered in combination to provide the larger information's in plant defense elicitation. Association of results from the different approaches, the early plant responses and the identified protein shown to be differentially expressed after aphid saliva application, is discussed in term of the development of efficient and fast ways to assess the role of plant defense elicitors such as the ones from insect sucking feeding insects.

PISTACHIO TREE RESPONSES TO PSYLLID CONTAMINATION THROUGH THE GROWING SEASON

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The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psylloidea) is an indigenous pistachio pest in Iran. It is now the most serious pest throughout the pistachio-producing regions of the country. Usually the psyllid population rapidly increases immediately after bud-break in early spring through to mid-autumn. This is a common pistachio pest in pistachio plantations, usually reaching outbreak levels. This insect does not cause deformation of the plant tissues e.g., leaf-rolling, gall induction, or by injection of toxin into feeding sites, and there are no records of it acting as a vector of plant diseases. However, the psyllid nymphs (in particular) ingest large amounts of the nitrogen-poor phloem sap to obtain their nutritional requirements and this result in the excretion of large amounts of concentrated honeydew of paste-like consistency, which becomes dry almost immediately after elimination. The sap removed by large populations of nymphs and adult psyllids causes severe problems during kernel development, with a subsequent bud drop and defoliation. This damage affects not only the yields in the current year but also in the two subsequent years. Field and laboratory studies have shown that there is an obvious difference in response levels between pistachio cultivars. The well-known commercial cultivars, such as Akbari and Kalah-qochi, appear to be the most attractive cultivars for *A. pistaciae*, whereas cultivars with low nut quality and the wild pistachio species with poor nut quality are significantly less susceptible. The response of the commercial cultivar Kalah-qochi to 4 different levels of psyllid nymph density, e.g., means of 25, 3.2, 1.8 and 0.5 nymphs per leaflet showed that, with decreasing nymphal density, the blanked nuts decreased from 53 to 27%, empty nuts declined from 23 to 7% and split nuts increased from 25 to 66%. In addition, under high nymphal density, bud drop and defoliation occurred. Therefore, the responses of the pistachio trees to *A. pistaciae* are closely related to the number of nymphs per plant, the timing of the invasion, duration of feeding, the fruit density and the cultivar. Defoliation at any time during the growing season causes serious losses, but bud drop almost always occur prior to defoliation. In sensitive cultivars, heavy infestations during the kernel development period, e.g., July and August, or even earlier in the season before endocarp development can cause complete defoliation after a few weeks.

HERBIVORY BY A PHLOEM-FEEDING INSECT INHIBITS FLORAL VOLATILE PRODUCTION

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Volatile organic compounds emitted by plants mediate an array of interactions with herbivores, pollinators and predators. Though there is extensive knowledge on the release of volatiles from vegetative tissue and release from flowers, very little is known on how they interact. We studied the effects of aphid and caterpillar damage to vegetative tissues on the emission of floral scents by *Sinapis alba*. Damage by the specialist aphid *Lipaphis erysimi* just prior to anthesis caused a near-total shutdown of volatile emission from flowers after 72 hours and more so after 96 hours. The generalist aphid *Myzus persicae* caused a large reduction, though not as important as *L. erysimi*. Caterpillars of the pyralid moth *Plutella xylostella* caused no reduction in floral volatile emission. There was a linear reduction in volatile emission with increasing *L. erysimi* density over 72 hours. When damage by *L. erysimi* started after anthesis no volatile shutdown was detected, so aphid damage only affects floral volatiles if it occurred before anthesis. Field observations showed no effect of this reduction on the total number of flower visits by pollinators. The responses of two aphid natural enemies, *Coccinella septempunctata* and the parasitoid *Diaeretiella rapae* were studied in the olfactometer to determine whether they detect changes in floral volatiles. *C. septempunctata* was attracted to the vegetative parts of *L. erysimi*-damaged plants, but preferred the flowers of undamaged plants. *D. rapae* on the other hand responded preferentially to the odour of flowers from damaged plants over the odour of flowers from undamaged plants. This is the first unequivocal demonstration of an effect of herbivory to vegetative parts affecting floral volatiles and raises a series of ecological questions in relation to indirect interactions between herbivores and pollinators.

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THE PAMP-TRIGGERED IMMUNITY RESPONSE IS INVOLVED IN PLANT DEFENSE RESPONSE TO APHID ATTACK AND IS SUPPRESSED BY AN APHID EFFECTOR

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Aphids are insects which feed on phloem sap using their stylets. As in plant-pathogen interactions, successful colonization of a host by an aphid is thought to involve effectors, which manipulate plant processes to enhance susceptibility to the aphid. These effectors are most likely salivary gland proteins that are secreted into the saliva and then introduced into the plant during aphid feeding. Many pathogen effectors target the plant Pathogen-Associated Molecular Pattern (PAMP) Triggered Immunity (PTI) pathway. We previously identified a salivary gland protein from the aphid *Myzus persicae,* Mp10, which suppresses the Reactive Oxygen Species (ROS) burst elicited by the PAMP flg22 (Bos, Prince *et al.,* 2010. PLoS Genetics 6(11): e1001216). Further investigation of Mp10 function revealed that this effector also suppresses the calcium burst that precedes the flg22 ROS burst, as well as the ROS burst elicited by crude aphid extract. Furthermore, crude aphid extract ROS burst was decreased on some but not all Arabidopsis mutants impaired in the PTI signaling pathway. Non-host (*Acyrthosiphon pisum*) aphids also performed better on these mutants, suggesting that specific PTI signaling components are involved in the perception of aphid elicitors. Further studies to investigate how Mp10 aids aphid performance are under way. In conclusion, our results so far indicate that PTI plays a role in plant defense response to aphid attack and is suppressed by an aphid effector.

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MOLECULAR BASIS OF HOST DEFENSE AGAINST GREEN PEACH APHID

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Aphids are phloem-feeding insects that are important pests of a wide variety of plants. Aphid feeding results in the removal of phloem sap and alterations in source-sink patterns, both of which limit plant productivity. In addition, several aphids also vector viral diseases. *Myzus persicae* (Sulzer), commonly known as the green peach aphid (GPA), is a polyphagous insect with a wide-host range that includes the model plant *Arabidopsis thaliana*. Arabidopsis utilizes antibiotic and antixenotic mechanisms to curtail GPA infestation. We have exploited this interaction between *Arabidopsis thaliana* and GPA to characterize the molecular basis of host defense against GPA. Our studies indicate that the *PAD4* (*PHYTOALEXIN-DEFICIENT4*) gene, which encodes a protein that is homologous to eukaryotic α/β fold hydrolases, is an important modulator of Arabidopsis defense against GPA. *PAD4* is required for deterring insect settling on the plant, and for limiting insect feeding from sieve elements and fecundity. In addition, *PAD4* is required for the activation of premature leaf-senescence that is activated in response to the infestation. *PAD4* expression is induced in response to GPA infestation. This increase in *PAD4* expression is modulated by the *TPS11* gene, which encodes an enzyme that synthesizes trehalose, a non-reducing disaccharide. Our studies are suggestive of a regulatory function for *TPS11* and trehalose metabolism in promoting defense against GPA. In addition to modulating *PAD4* expression, recent studies indicate that *TPS11* promoted reallocation of carbon into starch at the expense of sucrose, which is the primary plant-derived carbon and energy source for GPA, contributes to host defense against the insect. Arabidopsis also utilizes an additional antibiosis mechanism that involves the *MPL1* (*MYZUS PERSICAE-INDUCED LIPASE1*)-encoded lipase. This mechanism parallels the *PAD4*-dependent mechanism. Genes similar to *PAD4*, *TPS11* and *MPL1* exist in other plants, suggesting that their function in defense against GPA is likely conserved beyond Arabidopsis.

HEMIPTERAN FEEDING AND PLANT DEFENSE RESPONSES: FROM CELLULAR DESTRUCTION TO STEALTHY FEEDING

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In response to herbivore feeding, plants perceive signals generated in response to tissue damage and insect oral secretions to activate and/or suppress defense-signaling pathways. The balance of these signals varies depending on the mode of feeding. Within the order Hemiptera, there is a wide variety of feeding behaviors- from the destructive lacerate-and-flush strategies to the non-destructive feeding behaviors of whiteflies. Accordingly, plant molecular and biochemical responses to hemiptera vary with respect to the amount of tissue damage and their site of feeding. Plant defense strategies, particularly induced defenses, provoked by hemipteran feeding will be overviewed. The similarities and differences amoungst hemipteran insects and other arthropod herbivores will be highlighted. In many cases, defenses induced by herbivore attack protect the damaged plant against herbivory. However, it is clear some hemipterans engineer the gene expression programs of their host to provide a more suitable environment for insect development.

DISSECTING RESISTANCE TO APHIDS (*ACYRTHOSIPHON* SPECIES) USING THE MODEL LEGUME *MEDICAGO TRUNCATULA*

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Aphids, including bluegreen aphid (BGA; *Acyrthosiphon kondoi*), pea aphid (PA; *A. pisum*), and spotted alfalfa aphid (SAA; *Therioaphis trifolii f. maculata*) are important agricultural pests in legume agriculture. Australian breeders have introgressed BGA resistance into three popular cultivars and generated three new resistant lines in the model legume *M. truncatula*. Further characterization showed that each resistance line operates against a number of major legume aphid species. However, the magnitude of resistance varied depending on the *M. truncatula* line and/or aphid species. We have focused on one pair of near isogenic lines, A17 (susceptible) and Jester (resistant) in which single dominant genes condition resistance to BGA, PA and SAA. We have fine mapped two of these resistance genes called *AKR* (*Acyrthosiphon kondoi* resistance) and *TTR* (*Therioaphis trifolii* resistance) and the fine mapping of the third resistance gene termed *APR* (*Acyrthosiphon pisum* resistance) is underway. We have generated very near isogenic lines of *M. truncatula* A17 harbouring these aphid resistance genes, which are powerful tools that essentially eliminates background noise in our transcriptomics and metabolomics experiments. Using these resources, we are making considerable progress on deciphering downstream signalling and defence mechanisms against these aphid species including phytohormone profiling and the identification of transcription factors that may control a successful plant response to aphid attack.

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Section 5

Hemipteran-Plant Pathogen Interactions

REGULATION OF HOST SWITCHING AND TRANSMISSION IN *XYLELLA FASTIDIOSA*

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Xylella fastidiosa is a bacterium that causes disease in a large number of economically important crops. This bacterium is transmitted by xylem sap-feeding insects, primarily sharpshooter leafhoppers, in a persistent manner. *X. fastidiosa* colonizes both the xylem network of plants and the foregut surface of its insect vectors, where it forms a biofilm. A cell-cell signaling mutant of *X. fastidiosa*, deficient in production of the signaling molecule DSF, is unable to colonize vectors and is only poorly transmitted to plants. However, a mutant that generates a distinct profile of signaling molecules is transmitted by vectors, but apparently unable to form biofilms and has decreasing transmission efficiency over time. In addition to this density dependent gene regulation of transmission, environmental cues also affect vector colonization. Host switching from plants to insects is dependent on the presence of plant structural polysaccharides such as pectin, which up-regulates the expression of adhesins required for vector colonization. In addition, within insects, chitin can be used as a carbon source by *X. fastidiosa*, and its presence also leads to phenotypic changes in this bacterium, primarily associated with cell adhesion. We propose that density dependent signals and environmental cues are part of a complex regulatory network controlling *X. fastidiosa* host switching and vector colonization.

APHID - PHYTOVIRUS INTERACTIONS: INVESTIGATION OF VIRUS BINDING MECHANISMS IN INSECT VECTORS BY LECTIN USE AND PROTEOMIC APPROACH

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Aphids are well known for their role in virus transmission to host plant. In some cases, the virus is transmitted from plant to plant simply attached to the cuticule of the mouthparts or the forget. For other, circulative virus transmission based on virion internalization through the aphid gut followed by transfer to salivary glands and finally to next plant during aphid feeding is required. In both situations, presence of receptor components through the digestive tract of the aphids is needed for virus binding and further transmission to next plants even if not localized at the same place. In order to investigate the specific binding of virus on particular aphid receptors, two aphid-virus models were selected to be tested using several lectins showing differential sugar binding specificities. Virus transmission efficacy assays with *Myzus persicae* and potato virus but also *Acyrthosiphon pisum* and pea enation mosaic virus were performed using a range of lectins to assess the potential competition of lectins and virus. Some interesting lectins were found to reduce the virus transmission with a 2 fold factor showing potential use of lectin in virus spread control. The aphids were also investigated by a proteomic approach using a two Dimension-Differential In Gel Electrophoresis (2D-Dige) coupled with mass spectrometry to determine the aphid proteins involved in virus transmissions. Head or digestive tubes of aphids were collected and investigated for non persistant or persistant virus models respectively. Differential abilities of aphids to transmit the selected virus models are discussed in relation with lectin affinity specificity and investigated aphid proteins found to be involved in vector-virus interactions. The application of lectin as potential way to reduce virus transmission by aphids will also be developed.

THE EFFECT OF CYAZYPYR™ IN REDUCING HEMIPTERAN PEST-TRANSMITTED DISEASES IN CROP PLANTS.

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Hemipteran pest-transmitted plant diseases are currently causing severe yield and quality declines in multiple crops resulting in significant economic losses to growers. Insecticides are an important component in the management of insect-vectored diseases in agricultural systems. However, while most insecticides provide good to excellent control of the insects that vector plant diseases when applied correctly, they do not reduce disease transmission to satisfactory economic levels. Cyazypyr™ (a.k.a. DPX-HGW86 and cyantraniliprole), is a novel cross-spectrum second generation anthranilic diamide insecticide that was discovered by the DuPont Company, and is currently being commercialized for use in agricultural crop management systems and other pest management systems. Cyazypyr™ is the third molecule in the anthranilic diamide class of chemistry to be to be commercialized, but the first with significant efficacy and control of Hemipteran pests. It exhibits a novel mode of action, by selectively activating the ryanodine receptor in insect muscles, resulting in rapid cessation of feeding in affected pest insects. This causes reductions in the capability of the affected pest insects to vector plant diseases. The effect of Cyazypyr™ on viral and bacterial diseases transmitted by several Hemipteran (whiteflies, psyllids, and aphids) and non-Hemipteran insects will be discussed.

BEMISIA TABACI BIOTYPE B ACQUIRES, BUT DOES NOT TRANSMIT A PASSIFLORA ISOLATE OF *SIDA MOTTLE VIRUS*

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A disease named passion flower little leaf mosaic, caused by a begomovirus (family *Geminiviridae*), was first reported in *Passiflora edulis* f. *flavicarpa* in the State of Bahia, Brazil, by Novaes et al. (Plant Pathology, 648-654,2003). The virus was transmitted from field infected plants to healthy passion flower by an unidentified biotype of *Bemisia tabaci*, which was found colonizing these plants. It is worth to note that passion flower plants are not considered as host of whiteflies, and it is rare to observe them on these plants in Brazil. A begomovirus closely related to tomato-infecting isolates was identified after molecular characterization (Ferreira et al.,Plant Pathology,221-230,2009). Another passion flower plant infected with a begomovirus was later found in São Fidelis County, State of Rio de Janeiro, Brazil. It was infected with an isolate of *Sida mottle virus* (SiMoV), determined by full DNA-A sequence analysis, and referred here as SiMoV-P. The virus could infect *Nicotiana benthamiana*, *Solanum pimpinelifollium*, and *Passiflora morifolia* plants by particle bombardment and grafting. *P. morifolia* is more preferred by *B. tabaci* biotype B than *P. edulis* f. *flavicarpa* (Nunes et al., 2008), and it was chosen for transmission studies. Preliminary attempts to transmit SiMoV-P with *B. tabaci* biotype B failed. Therefore, the purpose of the present work was to further investigate the transmission of SiMoV-P by *B. tabaci* biotype B and to analyze the presence of the virus in the insect. An isolate of *Tomato yellow vein streak virus* (ToYVSV) was used as control for insect transmission tests. Adults of *B. tabaci* biotype B were fed during 24h in *P. morifolia* infected with SiMoV-P. Then, groups of 10 insects were transferred to each healthy *P. morifolia* for a 24 hours inoculation access period. The same procedure was applied for the transmission of ToYVSV to tomato plants. Inoculated plants were analyzed by symptom expression and virus detection by PCR with universal primers for begomoviruses. To improve the PCR detection, total DNA extracted from test-plants was first submitted to rolling circle amplification (RCA). Groups of 10 insects fed on *P. morifolia* and tomato plants infected with SiMoV-P and ToYVSV, respectively, were tested for virus detection by PCR. Other groups of insects were frozen in acetone and immediately dissected to separate their heads and prothoraxes from the rest of the bodies. Total DNA was extracted from each part separately and submitted to RCA followed by PCR for virus detection. None of the 20 *P. morifolia* plants inoculated with SiMoV-P by *B. tabaci* biotype B showed symptoms or tested positive for begomovirus by PCR. Tomato plants inoculated with ToYVSV showed symptoms and the virus was detected in all test-plants. Both begomoviruses were detected by PCR in adult whiteflies that had fed on infected *P. morifolia* and tomato plants, respectively. SiMoV-P and ToYVSV were also detected in the head/prothorax part and the rest of the body of the insects, suggesting that both begomoviruses were

acquired and circulated in the body of *B. tabaci* biotype B, but only SiMoV-P was not transmitted to the inoculated plants. Further studies are necessary in order to identify the reason for the failure of *B. tabaci* biotype B to transmit SiMoV-P.

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TRANSMISSION *IN VIVO* OF ELM YELLOWS PHYTOPLASMA (16SrV) BY *AMPLICEPHALUS CURTULUS* (HEMIPTERA: CICADELLIDAE) IN RYEGRASS (*LOLIUM MULTIFLORUM* CV. TAMA)

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Several leafhopper species are very important in the phytoplasma transmission and dispersion. Phytoplasmas are causal agents of a wide range of plant diseases around the world, being particularly important in Chile, current cases reported in sugarbeet, grapesvine, and Chilean shrubs with agronomic potential such as *Ugni molinae*. Current research has showed the presence of a phytoplasma of Elm Yellows group in *U. molinae, Lolium multiflorum* and the leafhopper *Amplicephalus curtulus*. With the aim to evaluate and confirm the transmission process from native plants to grasses by *A. curtulus*, nynphs of 5° instar were put in cage with *U. molinae*-phytoplasma infected (n = 3 plant per cage, 3 cages) for 72 h. After that, phytoplasma infected plants were replaced by no infected ryegrass plants (n = 7 leafhopper per plant, 5 plant per cage, 3 cage) for 20 days (24±2℃, 16:8 h, and 60-75 % RH) (latent period). These plants were replaced by new plants of ryegrass and were put in the cages for 14, 7, and 1 day (trasmission periods). Subsequently, these plants were taken off to other cages without insects and were maintained there for 30 days. Under the same conditions (but without insect) and an equal number of ryegrass plants were placed in other cages as a control treatment. DNA was extracted from insects and plants used in the latent and transmission periods, and controls. The DNA was amplified by direct (primers P1/P7) and Nested-PCR (primer F2n/R2). PCR products were sequenced directly. The 46, 60 and 13% of the plants exposed to infected *A. curtulus* by 14, 7 and 1 days, respectively, were inoculated with Elm yellows phytoplasma. Plants not exposed to *A. curtulus* or those plants that were in the latent period did not amplify phytoplasma. We show that *A. curtulus* has the ability to transmit phytoplasma from *U. molinae* to ryegrass, being an adecuate period of transmission seven days; less time could affect the incidence or the detection process, because the results indicate that latent period in this leafhopper is higher than 20 days at controlled conditions.

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DISTRIBUTION AND ABUNDANCE OF *EXITIANUS OBSCURINERVIS,* POSSIBLE VECTOR OF *SPIROPLASMA KUNKELII* IN MAIZE CROPS IN THE TEMPERATE ZONE OF ARGENTINA

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Spiroplasma kunkelii is the main causal agent of “corn stunt”, an important disease of maize crops, exclusively transmitted by members of the Cicadellidae family, with *Dalbulus maidis* being the most efficient and so far the only one reported in Argentina. In North America, members of Exitianus genus act as field transmitters of *S. kunkelii*. The aim of this study is to determine the distribution and abundance of E. obscurinervis in the temperate zone in Argentina, the largest corn production area in the country, and relate it to the presence of S. kunkelii. Systematic samplings were conducted during the period from the 2002/03 to 2008/09 crop years in the Argentine central farming area. These consisted of 50 sweeps of an entomological net, on maize, surrounding wild grasses and winter cereals. The diagnosis was made by DAS-ELISA in R3-R4 crops. In the horticultural belt around Córdoba city, the presence of E. obscurinervis was registered throughout the year, including during the crop susceptibility period between September and January. The maximum abundance during this period in all the crop years was 1 individual/sampling, except in 2007/08 when the maximum was 0.5 individuals/sampling. On the other hand, *D.maidis* populations were detected when the crop had escaped the susceptible period. Therefore, the high incidence of disease during 2003/04 (48%), with *D. maidis* absent during the susceptible crop period, might be explained by the presence of *E. obscurinervis* rather than that of *D. maidis*. In the temperate zone of Argentina, the presence of E. obscurinervis during the susceptible period as well as *S. kunkelii*, was detected as far as Saladillo (35.63º south latitude) but the presence of *D. maidis* during this period was only recorded as far as Suardi (30.53º south latitude). These results suggest that *E. obscurinervis*, or another member of the Cicadellidae family, might be playing an important role in *S. kunkelii* epidemiology, in the temperate area of Argentina
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EARLY MOLECULAR PATHWAYS TRIGGERED UPON PLANT-INSECT INTERACTION CAN BE USED BY VIRUSES TO IMPROVE TRANSMISSION

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The vast majority of plant viruses are transmitted by hemipteran insect-vectors. Although several modes of interaction between viruses and insect-vectors have been described, the most frequent is the so called “non-circulative transmission”. In this type of transmission, the virus particles are taken up within infected plants during feeding of the insect, and retained specifically on attachment sites locate within the stylets, or in some cases within the foregut. This specific binding to the insect anterior alimentary tract is most often highly labile, and the virus is rapidly released upon feeding of its vector on a new healthy plant. Detailed studies on the *Cauliflower mosaic virus* (CaMV), transmitted non-circulatively by several aphid species, have recently revealed two interesting phenomena:

-First, a cuticular protein is used as a specific receptor by CaMV, and we have recently shown that this protein is located exclusively in a distinct anatomical feature, lining the bottom of the bed of the common duct at the extreme tip of the maxillary stylets. This newly described anatomical structure is present in all aphid species investigated thus far, and has been named the “acrostyle”. We have shown that it contains an important concentration of cuticular proteins with a typical RR2 motif, representing potential candidates for the CaMV receptor. The physiological function of the acrostyle, its role in the aphid-plant interaction, as well as in the vector-transmission of other non-circulative plant virus species remains elusive.

-Second, preliminary evidence indicates that CaMV can high jack very early steps of the signal transduction cascade induced when the host plant perceives the feeding activity of an aphid. Indeed, the aphid puncture into an infected leaf is inducing a sudden change in the composition and morphology of the CaMV transmission body (TB), an inclusion body known to regulate the virus acquisition by the aphid. These changes in the TB are occurring within seconds, a time lap compatible with the duration of the aphid intracellular punctures, and are positively correlated with a dramatic increase in the transmission rate. To date, the nature of the initial stress triggered by the aphid and perceived by the plant is unknown, the signal transduction cascade is not identified, and the molecular mechanisms translating this signal into an increased transmission are even more mysterious. Both these phenomena will be presented at the meeting, and a particular emphasis on one or the other will depend on the respective progress on each line of research.

PLANT AND APHID PARTNERS OF POLEROVIRUSES: ROLE IN VIRUS TRANSMISSION BY APHIDS?

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Poleroviruses are phloem limited viruses strictly transmitted by aphids in a circulative and non propagative manner. Virions are acquired by aphids when ingesting sap from infected plants. Virus particles cross the gut epithelium and the accessory salivary gland cells before being released, together with saliva, into the plant during a subsequent feed. This highly specific transcytosis mechanism relies on the presence of virus receptors on the surface of the aphid cells. We developed several approaches to identify virus partners in the plant and in the aphid to analyse their role in virus transmission by the vector. By screening different aphid cDNA libraries using a yeast two hybrid system, only few candidates were able to bind virus structural proteins. Among them, we found two nuclear proteins (GAR1 and ALY) which may not be the true virus-receptors but could be considered as virus-sensors. An Ephrin receptor-like protein was also found to interact with the viral proteins. Involvement of these candidates in virus transport through the aphid needs to be analyzed by developing in the insect RNAi-based techniques. These experiments are in progress. We also looked for plant virus-partners and identified several phloem proteins able to bind purified virions *in vitro*. We showed that these proteins could stimulate virus transmission by aphids when added together with purified virus to the aphid diet (Bencharki et al. 2010, M.P.M.I., 23: 799). By developing a yeast two hybrid system using a phloem specific cDNA library, we identified five additional proteins able to bind viral proteins. Among them, we found ALY proteins already identified as aphid virus-partners suggesting that orthologous plant and aphid proteins could be implicated in the virus cycle. So far, a direct implication of these proteins in aphid transmission has not been observed and experiments are on going to analyze their functions.

DETERMINATION OF PVY-TRANSMISSION EFFICIENCIES OF DIFFERENT APHIDS SPECIES: A NEW APPROCH

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Non-persistent viruses, transmitted by a broad range of aphids including transient non-colonizing ones, are of main concern in the context of crop production as their epidemics can cause substantial economic losses. Relative efficiency factor (REF), which allows comparison of aphid vectoring efficiencies between species, is frequently used in literature. However, in the *Potato virus Y* pathosystem, reported REFs show strong variations according to the different studies. The aim of this study was to develop an alternative methodology to optimize the REF assessment. *In vitro* micropropagated potato plantlets were used as target plants to get phenotypically and genetically homogeneous material and minimize the bias due to plant defence response. Species-specific acquisition access period (AAP) (i.e. the time elapsed from the aphid contact with the infected plant until the first intracellular puncture) on a PVY-infected plant was assessed for each aphid species using electrical penetration graph technique (EPG). Finally, aphid clones were used to minimize intraspecific variability. EPG monitoring of aphid probing behaviour showed highly variable AAPs between the different aphid species. Shortest AAPs were obtained for *M. euphorbiae* and *M. persicae* (15 and 11 min, respectively) whereas *R. padi, S. avenae, B. brassicae* and *A. pisum* exhibited AAPs 30 min longer. The transmission rate obtained for *M. persicae* (83.3 %) was higher than the reported one in the literature. REFs assessment showed *A. pisum* and *B. brassicae* were poor efficient vectors while *M. euphorbiae* and *S. avenae* seemed to be efficient ones even though their respective REF were significantly lower than that of *M. persicae*. Regarding *R. padi*, *A. fabae,* they did not transmit PVY as they took the longest time to perform an intracellular puncture.
The hypothesis consisting in a compensation of a weak PVY-transmission efficiency by a higher number of vectors was assessed for *M. euphorbiae* and *S. avenae*. Results did not corroborate such hypothesis. We discussed about the use of this new methodology for REF evaluation and the need to consider aphid behaviour for such assessment.

HOST-PLANT DETERMINES THE PHYTOPLASMA ACQUISITION AND TRANSMISSION COMPETENCE BY LEAFHOPPER VECTORS

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Phytoplasmas are wall-less bacteria transmitted by hemipteran vectors in a persistent, propagative manner. Insect feeding preferences have a major role in transmission specificity. Vector insects can be polyphagous, oligophagous or strictly monophagous according to their ability to feed and reproduce on many, few or one host plant, respectively. Similarly, phytoplasmas may be generalists, infecting several different plant species, or specialists, infecting one or a few related plant species. We report on the differential capabilities of leafhopper vectors to acquire and transmit two phytoplasma strains (Chrysanthemum Yellows, CYP, "*Candidatus* Phytoplasma asteris") and (Flavescence dorée, FDP, "*Ca.* P. vitis"), following feeding on different plant species. Acquisition and transmission efficiencies of CYP by *Macrosteles quadripunctulatus*, *Euscelidius variegatus,* and *Euscelis incisus* vary dramatically according to the host-plant. Similarly, *Scaphoideus titanus* acquires FD with higher efficiency when feeding on broad bean compared to grapevine, its natural host-plant. A comparative analysis of CYP transmission competence by *Empoasca decipiens* and *E. variegatus* on daisy and broad bean plants also provides evidences of the role of the host-plant in phytoplasma transmission. A different feeding behavior of of vectors on different host-plants and/or different phytoplasma titers in different plant species can explain the influence of host-plant species in phytoplasma transmission. We discuss relationships between vector transmission efficiency and phytoplasma titer in the source plant (as determined by quantitative real time PCR assays) in daisy, broad bean and two different grapevine varieties, Barbera and Nebbiolo.

WHITEFLY VECTOR (*BEMISIA TABACI*) PROTEOME ELUCIDATION: FIRST STEPS TOWARD UNRAVELING THE COMPLEXITY OF WHITEFLY-BEGOMOVIRUS INTERACTIONS

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The whitefly *Bemisia tabaci* (Gennadius) sibling species group is one of most important arthropod vectors of plant viruses. Among these are the widespread and damaging genus, *Begomovirus*, family, Geminiviridae. While much research has been focused on whitefly-vector interactions at the level of transmission behavior, less is known about molecular, cellular, or genomic level interactions. To elucidate proteins (genes) essential to the begmovirus-whitefly transmission pathway we are characterizing the adult whitefly, alimentary canal, and salivary gland proteins at the proteome and/or transcriptome levels for *B. tabaci* biotype B, with a particular focus on those that interact directly and indirectly with virions during whitefly-mediated transmission. A total of 12 mass spectrometry runs were performed on total proteins extracted of the adult (i) whole whitefly (9 MudPIT's) and (ii) whitefly gut (3 MudPIT's), respectively. Protein preparations were digested with trypsin and subjected to LC-LC-MS/MS to determine each peptide spectra using Sequest and X! Tandem software. Scaffold (ver. 2_05_01) was used to validate MS/MS peptides and perform protein identifications, with a minimum of two peptides required for a valid protein call. The resultant proteins were identified using three different 'search databases' (dbs): (i) whitefly ESTs (sequenced, assembled, and annotated in PAVE by our group), referred to as whitefly version 4 ('WFV4') that contains 774,065 translated ESTs), (ii) a set of translated ESTS from ~12 insect species selected from the available insect sequence databases, herein, 'Select Insect' (Sl-Ins), and (iii) all proteins of all organisms of the class 'Insecta' (Insecta). The latter two databases were downloaded to the The University of AZ supercomputer (last download 03.11)**.** A total of 597 unique whitefly proteins were identified from the 12 MudPIT runs. Over 500 unique proteins were identified from the 'whole whitefly' preparation (9 MudPITs; 2,145 unique peptides; 35,663 spectra), whereas, whitefly gut preparations alone yielded 411 unique proteins (3 MudPITs; 1484 unique peptides; 14,703 spectra). Using the combined 'SI-Ins' and 'Insecta' dbs as the search databases, 245 whole whitefly and 166 gut protein hits were identified. Protein hits (gi numbers) from the three databases were imported into the Uniprot batch retrieval system to facilitate identification of top hits to organism and insect species, and based on GO Slim, KEGG, and Pfam descriptions. Using the combined 'SI-Ins' and 'Insecta' dbs, pea aphid (Hemiptera: s.o. Homoptera: Aphididae) sequences provided the greatest number of hits, followed by fruit fly (Diptera: Drosophilidae), and then

body louse (Phthiraptera: Pediculidae). In contrast, both the ‘WFV4’ db, and the batch retrieval from Uniprot had the greatest number of hits to fruit fly, followed by many other species, and then yellow fever mosquito. The top protein hits were dominated by predicted (61) and putative (59) hits in the combined ‘Sl-Ins-Insecta’ dbs, whereas, putative hits (42) dominated for the ‘WFV4’ db. Overall the extent of shared homology between the whitefly proteins and ESTs/proteins in the three search databases was expected to be highest for other homopteran sequences, namely, the pea aphid, which is the best represented homopteran to date. This pattern was observed for the ‘Sl-Ins’ and ‘Insecta’ dbs annotations, but not for the ‘WFV4’ db, an observation that likely reflects the less than optimal number of annotated ESTs in ‘WFV4’ database, even though it contains ~775,000 ESTS (15,045 contigs and 39,864 singletons) and whitefly transcripts share 100% homology with whitefly proteins. Further only 17% of whitefly transcripts in ‘WFV4’ had a top hit against a Uniprot accession. These results underscore the need for additional functional genomics projects for plant virus vectors and other homopterans that transmit plant pathogens.

ASSOCIATION BETWEEN *MAL DE RÍO CUARTO VIRUS* (MRCV) TITER AND TRANSMISSION EFFICIENCY BY 1st AND 3rd INSTAR NYMPHS OF *DELPHACODES KUSCHELI*

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MRCV causes the most important disease of maize in central Argentina. It is only transmitted by planthoppers (Hemiptera, *Delphacidae*) in a persistent propagative manner, being *D. kuscheli* the main vector species. Under controlled conditions, only 30% of MRCV-infected insects can actually transmit the virus. In turn, when MRCV is acquired by 1st instar nymphs (N1) the transmission frequency is higher than when acquired by 3rd instar (N3) nymphs. The aim of this work was to study the relation between N1 and N3 transmission efficiencies, and MRCV titer. Thirty *D. kuscheli* nymphs of each life stage were analyzed by 1:1 transmission tests (1 planthopper: 1 wheat seedling) with acquisition, latency and inoculation periods of 2, 17 and 1 day respectively. Absolute quantification of a fragment of MRCV segment 3 (MRCV-S3) in each planthopper was performed by qPCR in triplicate reactions using 1 ul of cDNA as a template and 200 nM of each primer in a final volume of 20 ul of SYBR Green. The Ct value obtained for each sample was extrapolated to a standard curve previously built from serial dilutions of a MRCV-S3 DNA fragment. Results were statistically analyzed by Mann-Whitney test. The N1 group showed higher transmission efficiency (60%) than N3 (28%). Regarding qPCR quantification, 20% and 26% of N1 and N3, respectively, resulted negative. All transmitting insects were positive and showed significantly higher viral titers than non-transmitting ones ($P < 0.003$ in N1 and $P < 0.013$ in N3). Our results showed that MRCV-transmitting planthoppers contained higher viral titers than non-transmitting ones, suggesting that successful transmission depends on a viral accumulation thershold. Thus, the higher transmission efficiency of N1 group might be explained by the greater number of individuals overcoming that threshold and consequently behaving as vectors. This work is relevant to better understand the mechanism underlying viral transmission and its epidemiological implications.

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TARGETING THE KEY PROTEIN RESPONSIBLE FOR INSECT MEDIATED VIRAL TRANSMISSION: AN APPROACH TOWARDS RESISTANCE DEVELOPMENT IN PLANTS

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Hemipteran group of sap sucking insect pests namely, *Nephottetix sp., Lipaphis erysimi*, *Aphis craccivora*, *Bemesia tabaci* and *Myzus persicae* severely damage many important food crops including rice and mustard not only by extracting the plant's nutrition but also transmitting disease causing viruses. Due to their unique feeding behaviour with piercing mouthparts their control has been difficult. Some mannose binding lectins from *Allium sativum* leaf (ASAL) and *Colocasia esculenta* tuber (CEA) have shown detrimental effects on the growth and development of a wide range of sap sucking insect pests. ASAL was further expressed in rice under the control of CaMV35S and phloem specific promoters and the efficacy of rice transgenics was monitored on the performance of *Nephottetix sp*, commonly known as green leafhopper (GLH) as well as *Nilaparvatha lugens* (BPH). GLH resistant T_1 ASAL rice plants were further evaluated for monitoring the incidence of tungro disease, caused by co-infection of GLH vectored Rice tungro bacilliform virus (RTBV) and Rice tungro spherical virus (RTSV). Surprisingly, the transgenics after artificial inoculation with viruliferous GLH did neither show any disease symptom nor could provide significant viral titre. The results to be presented are novel findings about such resistance development against the infection of RTBV/RTSV in ASAL expressing transgenic rice plants. Incidentally, ASAL has previously shown to have specific interaction with the typical insect borne "symbionin" protein having significant role in insect mediated virus transmission that opens a new avenue towards developing virus resistance in economically important crops.

COMPARATIVE ANALYSIS OF ELECTRICAL PENETRATION GRAPHS OF NYMPHS AND ADULTS OF *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) ON CITRUS

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The phloem-limited bacterium associated with citrus huanglongbing (HLB), *Candidatus* Liberibacter asiaticus, is transmitted by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) with a higher efficiency when acquired by psyllid nymphs. In this study, we compared electrical penetration graphs (EPG) of nymphs and adults of *D. citri* in citrus to investigate possible differences in probing activities that could explain the higher acquisition efficiency of the pathogen by nymphs. Fifteen health laboratory-reared nymphs (4^{th} instar) and adults (1-week old) were recorded for 5 h on health seedlings of *Citrus sinensis* (L.) Osbeck, using a DC-monitor, Giga-8 model. The same EPG waveforms previously described for *D. citri* adults were observed in nymphs. No significant differences were observed regarding the mean number of probes and waveforms C (representing pathway stylet activities), D (phloem contact), E1 (putative salivation in sieve tube elements) and E2 (phloem sap ingestion) performed by nymphs and adults in 5 h. Only 1 nymph and 3 adults (out of 15 tested) performed waveform G (xylem phase), showing that both stages are primarily phloem feeders. Mean waveform duration per event also did not vary between nymphs and adults, except for E1, which was longer in nymphs (mean of 175 s) than in adults (78 s). Both nymphs and adults started probing in a few minutes (averages of 1.2 and 2.7 min, respectively) after placed on the plant. The mean time to start phloem sap ingestion (E2) from onset of the first probe was slightly shorter in nymphs (62.7 ± 15.8 min, varying from 16.0 to 241.4 min), but not statistically different from adults (75.0 ± 13.2 min, ranging from 12.1 to 216.1 min). Overall, this study shows no substantial differences in probing behavior between *D. citri* nymphs and adults that could account for the higher acquisition efficiency of *Ca*. L. asiaticus by nymphs. It indicates, however, that some nymphs and adults can reach phloem sieve elements (and possibly acquire or inoculate the pathogen) in relatively short access periods (≥15 min).

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IDENTIFICATION OF LEAFHOPPER VECTOR PROTEINS SPECIFICALLY INTERACTING WITH PHYTOPLASMA ANTIGENIC MEMBRANE PROTEIN

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Phytoplasmas are uncultivable phloem-limited phytopathogenic wall-less bacteria and are associated with severe plant diseases spread worldwide. They are transmitted in a persistent propagative manner by phloem-sucking hemipteran insects. Due to the lack of cell wall, phytoplasma membrane proteins are in direct contact with hosts and are presumably involved in determining vector specificity. Such a role has been proposed for phytoplasma plasmid-encoded transmembrane proteins and for the major phytoplasma antigenic membrane protein (Amp). The aims of our work were to discover vector proteins interacting with Amp and to investigate their role in transmission specificity. Following controlled transmission experiments, four hemipteran species were identified as vectors of the "*Candidatus* Phytoplasma asteris", CYP strain, and three others as non vectors. Interactions between a recombinant CYP Amp and insect proteins were analysed by far Western blots and affinity chromatography. Amp specifically interacted with few membrane proteins from vector species only. Among Amp-binding vector proteins, actin and ATP synthase α and β subunits were identified by mass spectrometry and Western blots. Immunofluorescence confocal microscopy and Western blots on cell membrane and mitochondria fractions confirmed the localisation of ATP synthase, a known mitochondrial protein, in plasma membranes of midgut and salivary gland cells in the vector *Euscelidius variegatus*. Phytoplasma Amp is likely to play a crucial role in insect transmission specificity. The vector-specific interaction between phytoplasma Amp and insect ATP synthase is demonstrated for the first time. Plasma membrane ATP synthase is known as receptor for various ligands in vertebrates and arthropods. Intriguingly, phytoplasmas lack ATP synthesis genes: exploitation of host ATP synthetic machinery maybe required for phytoplasma survival or adhesion before entry in vector organs. This work also confirms the role of host actin, likely involved in internalization and intracellular motility of phytoplasmas, as shown for other intracellular pathogens.

MONITORING OF THE APHID FAUNA OF VECTOR-BORNE VIRUSES IN A NEWLY INTRODUCED YELLOW PASSION-FRUIT COMMERCIAL CROP

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Brazil is the world's largest producer of passion-fruit (*Passiflora* spp.) with a production of 492 thousand tons yearly. The "woodiness disease", induced by *Cowpea aphid-borne mosaic virus* (CABMV), is the most important viral disease affecting this crop in Brazil, and has led producers to migrate to new regions or to abandon its cultivation. CABMV induces fruit woodiness, causing loss of production and economic losses. Both CABMV as well as the *Cucumber mosaic virus* (CMV, *Cucumovirus*) are reported in passion-fruit, and transmitted by several species of aphids in a non-persistent manner. For twelve months (May/2010 to April/2011), in an area of 1,000 m^2, characterized by the recent introduction of passion-fruit cultivation, in the municipality of Pinhalzinho (22°40'55"S/46°40'51"W, altitude of 750 m), state o f São Paulo, virus-host-vector interactions were monitored in an orchard with 200 plants of yellow passion-fruit (*P. edulis* f. *flavicarpa*) cultivar IAC-275. The virus detection in passion-fruit and weed species was monitored monthly, through PTA-ELISA with polyclonal antiserum against CABMV and CMV. To determine the abundance of aphids, we used yellow sticky and Moericke-type traps in the field. Aphids captured were counted and identified under a stereomicroscope and using a dichotomous key. In July [winter with temperature average of 10°C (T_{min}), 26°C (T_{max}) and rainfall of 31.8 mm], there was a greater abundance and diversity of species, prevailing *Aphis fabae*, *A. gossypii*, *Toxoptera citricidus*, *Uroleucon ambrosiae* and *Pemphigus bursarius*, while *A. craccivora*, *A. nasturdii*, *A. spiraecola*, *Aulacorthum solani*, *Macrosiphum euphorbiae*, *Myzus persicae* and *T. aurantii* were characterized as secondary. In January [summer with temperature average of 18°C (T_{min}), 29°C (T_{max}) and rainfall of 521.3 mm], we found a lower diversity, recording *A. fabae*, *A. gossypii*, *T. citricidus*, *U. ambrosiae*, *P. bursarius* as the most abundant and *Pentalonia nigronervosa* as secondary species. Population density of aphid fauna flying over the crop was estimated at 99,000 aphids/1,000 m^2 in winter and 89,700 aphids/1,000 m^2 in summer. In this period, it was not detected the presence of virus in the passion-fruit plants, but the weeds *Bidens pilosa*, *Crotalaria* sp. and *Nicandra physaloides* behaved as reservoirs of CMV. Despite the pressure of virus and vectors in the region monitored, it was concluded that the transfer of passion-fruit crops for new areas, with sporadic source of virus inoculums, can be a recommended practice to delay the entry of CABMV and CMV.

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BEMISIA TABACI SECONDARY SYMBIONTS: THEIR FUNCTIONAL ROLES IN VIRUS TRANSMISSION AND WHITEFLY BIOLOGY

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The whitefly *Bemisia tabaci* is an extremely devastating insect pest that harbors several symbiotic bacteria, including *Portiera aleyrodidarum*, the primary obligatory symbiont, as well as several secondary symbionts, including *Rickettsia*, *Hamiltonella*, *Wolbachia*, *Arsenophonus*, *Cardinium* and *Fritschea*, the function of which is unknown. The distribution of these secondary symbionts is biotype-dependent. In Israel, the B biotype harbors *Hamiltonella* while the Q biotype harbors *Wolbachia* and *Arsenophonus.* Both biotypes harbor *Rickettsia.* Examples of ongoing studies in our lab, aimed at deciphering functional roles for some of these secondary symbionts in their whitefly host will be presented. A first example includes the recent finding that a GroEL protein produced by *Hamiltonella* in the B biotype, but not other GroELs from other symbionts in the Q biotype, interacts with, and safeguards the *Tomato yellow leaf curl virus* (TYLCV) while circulating in the whitefly's body. This interaction contributes to the ability of the B biotype to be a much more efficient TYLCV vector than the Q biotype that lacks *Hamiltonella.* Other examples include the involvement of *Rickettsia* in the response to stress including chemical insecticides and heat shock, and the unique distribution of *Rickettsia* in the pathway of circulative viruses including stylets, midgut, hemolymph and salivary glands, its horizontal transmission between the reproductively isolated B and Q biotypes through the plant host, and its rapid establishment in *Rickettsia*-free whiteflies. Such unique interactions and possible roles for *B. tabaci* secondary symbionts might be employed in developing novel strategies for whitefly control.

EFFECT OF THE BEGOMOVIRUS TRANSMISSION EFFICIENCY FOR THE PREDOMINANCE OF *TOMATO SEVERE RUGOSE VIRUS* (TOSRV) IN TOMATOS CROPS

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Tomato severe rugose virus (ToSRV) and Tomato golden vein virus (TGVV) are bipartite begomoviruses, transmitted by whiteflies (*Bemisia tabaci* biotype B), frequently found infecting tomato (*Solanum lycopersicum*) crops in Brazil. To more fully understand the interaction begomovirus/whitefly, as well as how occurrence of mixed infection may influence virus emergence and dominance in an infected plant, a study on begomovirus transmission was carried out focusing on the determination of transmission efficiency of two begomoviruses, ToSRV and TGVV, in single and double infection. Transmission tests were done with single and double infections, using the ToSRV 1164 and TGVV 1799 isolate. Whitefly colonies were maintained on virus-free cabbage or tobacco plants in a greenhouse. The virus isolates were inoculated on tomato plants ca. 30 days prior to transmission tests, when they showed clear interveinal chlorosis and leaf distortion symptoms. Infection was confirmed by PCR using species specific primers. A large population of whiteflies was allowed to mass feed on virus-infected source plants for 48h acquisition access period (AAP). Following virus acquisition, the whiteflies were transferred to plastic cups (three insects per plant both in simple and mixed infection) and allowed an inoculation access period (IAP) of 48h. After inoculation, the insects were manually killed. Plants were incubated for three weeks and analyzed for the presence of ToSRV and TGVV by PCR. The percentage of infected plants (detection by PCR) by ToSRV and TGVV in single infections was respectively equal to 41.9 and 25.0%, and in mixed infection it was 86.2% for ToSRV, 3.6% for TGVV, and 8.1% for both viruses. After 48 h AAP, the insects used in the transmission tests were collected, total DNA was individually extracted and PCR done to assess the percentage of insects that have acquired ToSRV and TGVV (single and mixed infections). Almost 90% of whiteflies acquired ToSRV or TGVV in single infection and all evaluated insects acquired both viruses when fed on leaves with mixed infection. A second trial was carried out for testing the effect of mixed infections, by using large cages, and in two types of mixed infection: in the first type, one tube with ToSRV viruliferous insects and one with TGVV were placed inside a cage containing 12 healthy plants; in the second type, viruliferous insects (two tubes) fed on detached leaves of plants with mixed infection were caged with the healthy plants. As a result, by inoculating both viruses from singly infected viruliferous whiteflies 32.7% of the plants were infected with ToSRV, 5.3 by TGVV and 27.4 by the two viruses. When the virus source was a plant infected with both viruses, 100% of infected plants contained only ToSRV. Therefore, when the two viruses were inoculated with the same insect, TGVV failed to infect any plant, but when the two viruses were

inoculated by different insects, there was a higher infection rate of TGVV. We concluded that as the percentage of whiteflies that acquired ToSRV and TGVV in single and mixed infections was similar, the highest rate of ToSRV infection seemed not to be related to differences in the acquisition of virus by the insect vector. Moreover, in all tests, the transmission efficiency of ToSRV was higher than of TGVV. This was an expected result and may explain why ToSRV predominates over TGVV in the field.

CAULIFLOWER MOSAIC VIRUS USES THE PLANT HOST CELL TO SENSE THE APHID VECTOR AND OPTIMISE ITS OWN TRANSMISSION

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Transmission of Cauliflower mosaic virus (CaMV) by aphids depends on the presence of viral inclusions, the Transmission Bodies (TB), in infected plant cells. TB contain the aphid transmission factor, the viral protein P2, and the viral protein P3. When TB do not form, no transmission occurs even when infected cells contain functional P2 (Khelifa et al. 2007, J. Gen. Virol., 88: 2872). Thus, TB are structures specialised for transmission, hence our interest to study their formation and function (Martinière et al. 2009, Plant Journal, 58:135). We detected that stress induces import of apparently soluble tubulin into TB. FRAP experiments indicated a high turnover rate of TB-contained tubulin. In aphid transmission experiments, we found that aphids fed on stressed infected leaves transmitted CaMV better than aphids fed on control leaves, that there was a positive correlation between tubulin entry in TB and transmission efficiency, and that aphid punctures themselves might induce rapid (within seconds) tubulin influx into TB. The Ca^{2+} ionophor A23187 induced tubulin influx into TB; the Ca^{2+} channel blocker La^{3+} completely inhibited transmission. The microtubule depolymeriser oryzalin inhibited transmission indicating involvement of microtubules in CaMV transmission. Finally, incubation of infected protoplasts with NaN_3 induced disintegration of TB and relocalisation of P2 and virions on microtubules, concomitant with drastically increased CaMV transmission. Preliminary data indicate that also ROS might induce TB disintegration. Taken together, our results indicate that a Ca^{2+} signalling cascade, which might be triggered as an early plant defence response to exploratory intracellular stylet punctures of the aphid vector, “activates” the otherwise “dormant” TB for transmission by causing massive entry of tubulin in TB, possibly followed in a second step by redistribution of P2 and virions on microtubules all over the cell. Thus it seems that CaMV deflects host perception and signalling pathways to perceive the presence of the aphid vector and to actively prepare its own acquisition.

DO ALL NONCIRCULATIVE APHID-TRANSMITTED VIRUSES SHARE THE SAME RETENTION SITES?

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Non-circulative (NC) aphid-transmitted viruses are known to be retained in a specialized anatomical structure of the common duct of the maxillary stylets and inoculated during successive intracellular stylet punctures (pd) in the epidermal and mesophyll cells. Different molecular virus-vector interactions mediate the retention and relationships between NC viruses and their specific receptors in the vector stylet cuticle. Some NC viruses, use the capsid protein (CP) to bind directly to the receptor in the aphid's stylets (Cucumoviruses), while others need virus encoded proteins that act as a bridge between the CP and the aphid's receptor. This is the case of Potyviruses, where the presence of the Helper component protein (HC-Pro) is mandatory for transmission and of the Caulimoviruses which require two additional proteins (P2 and P3). However, there is no evidence that all the above-mentioned NC viruses share the same retention sites in its aphid vectors causing competition or interference between each other when acquired together or one after the other. The possible competition for aphid's retention sites between different types of NC viruses was evaluated in a series of sequential transmission assays conducted with three types of NC viruses that follow different transmission strategies: *Turnip mosaic virus* (TuMV, *Potyvirus*) versus *Cauliflower mosaic virus* (CaMV, *Caulimovirus*), and *Cucumber mosaic virus* (CMV, *Cucumovirus*) versus *Zucchini yellows mosaic virus* (ZYMV, *Potyvirus*). The experiments were conducted on turnip plants and *Brevicoryne brassicae* L. as a vector of the TuMV-CaMV combination and on melon plants and *Aphis gossypii* Glover as a vector of the CMV-ZYMV combination. Results showed that a short acquisition access time of CaMV does not interfere with the subsequent acquisition and retention of TuMV, and both viruses are acquired, retained and transmitted concurrently by the same aphid. However, after long CaMV acquisition access periods the probability of retention and subsequent transmission of TuMV as well as the number of co-infected plant was remarkably reduced. On the other hand, no reduction of ZYMV transmission rate is observed when CMV is acquired previously. However, when ZYMV is acquired first, there is a significant reduction in the retention and subsequent transmission of CMV. We will discuss on the basis of our findings how a precise anatomical structure of the common duct of the aphid's maxillary stylets may host the same retention sites for different groups of NC-viruses.

TRANSMISSION OF *LETTUCE INFECTIOUS YELLOWS VIRUS* IS DETERMINED BY A VIRUS CAPSID PROTEIN MEDIATED VIRION RETENTION MECHANISM IN THE FOREGUT OF WHITEFLY VECTORS

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Members of the emerging and economically important genus *Crinivirus* are transmitted in a non-circulative manner by specific whitefly vectors via ill-understood mechanisms. Non-circulative transmission refers to the fact that upon acquisition, virions do not circulate through, replicate, or invade the salivary glands of the insect vector, before they can be inoculated into a plant. Plant infection by criniviruses occurs exclusively in the phloem and virions can only be acquired by the vector during prolonged feeding. Once, acquired, virions can persist in the vector from hours to days, but lose their ability to be transmitted when the vector molts. Among criniviruses, transmission of *Lettuce infectious yellows* (LIYV) by the whitefly *Bemisia tabaci* biotype A is best studied. However, little is known about the fate of the virus once it enters the vector. We have been investigating virus-vector interactions associated with the whitefly transmission of LIYV using an immunofluorescent localization approach in which virions or recombinant virus capsid components and reacting antibodies are acquired by live whiteflies. Our studies revealed that fluorescent signals, indicating the retention of virions, were specifically localized in the cibarium or foreguts of whitefly vectors but not within those of whitefly non-vectors. Moreover, the specific retention of virions strongly corresponded with the whitefly transmission of the virus. When four recombinant (*r*) LIYV capsid components were individually acquired by whitefly vectors, only the recombinant minor coat protein, *r*CPm, was retained in a significantly higher number of individuals. Notably, a CPm (transmission defective) mutant was defective in specific virion retention, whereas the CPm restored virus showed wild-type levels of specific virion retention and transmission. Taken together, these data suggest that the transmission of LIYV is determined by a CPm-mediated virion retention mechanism in the cibarium or foreguts of whitefly vectors.

DIRECT AND INDIRECT VIRUS EFFECTS ON THE PROBING AND FEEDING BEHAVIOR OF *APHIS GOSSYPII* GLOVER

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Virus infection may indirectly affect the performance and behavior of their insect vectors because of changes in the physiology and biochemistry of the infected host plant. Viral infection has been reported to cause changes in the host plants that may increase the attractiveness and preference to vectors, modifying the pattern of spread of the virus. However, all these changes may depend on the kind of plant virus-vector relationship. Conversely, direct effects of plant viruses on the behavior of their aphid vectors have never been reported, but they may be an important component in understanding plant virus epidemics. In the present work, we have studied the plant-mediated indirect effects caused by virus infection with the nonpersistent *Cucumber mosaic virus* (CMV) on the feeding behavior of the melon aphid, *Aphis gossypii*. Non-viruliferous aphids were connected to a DC-EPG device and placed on CMV-infected or non-infected cucumber plants for six hours. Furthermore, we have used the persistent virus *Cucurbit aphid-borne yellows virus* (CABYV) to study the feeding behavior of CABYV-viruliferous and non- viruliferous aphids on healthy cucumber plants to assess any direct effects of the virus on the feeding behavior of *A. gossypii*. Our results showed that CMV infection had a clear impact on the stylet activities of *A. gossypii* at the phloem level. Aphids rejected CMV-infected plants as a feeding source, and stayed significantly longer on sustained phloem ingestion activities in healthy than on CMV-infected cucumber plants. These results suggest that CMV-infection alters the behavior of aphids in a way that virus acquisition and retention is optimized and ideally adapted to a typical non-persistent virus transmission strategy.

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LATENT PERIOD OF *CANDIDATUS* LIBERIBACTER ASIATICUS IN *DIAPHORINA CITRI*

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Candidatus Liberibacter asiaticus (LAS) is the prevalent liberibacter species associated with citrus huanglongbing (HLB), one of the most devastating diseases for citrus production worldwide. The bacterium is transmitted by the Asian citrus psyllid, *Diaphorina citri* kuwayama (Hemiptera: Psyllidae). After acquisition from infected plants a latent period is required before inoculation of the pathogen in healthy plants. However, previous reports of latent periods for LAS are inconsistent, with durations ranging from 1 day to 3 weeks. Considering the importance of this information to understanding transmission mechanisms and HLB epidemiology, we conducted an experiment to examine the latency of LAS after acquisition by *D. citri* nymphs or adults. Groups of 1st instar nymphs and 1-week old adults obtained from a healthy laboratory colony were confined on LAS-infected sweet orange plants for a 48-h acquisition access period (AAP) at 25^{o}C. Next, 25 insects of each age group were individually and serially transferred to healthy sweet orange seedlings for successive inoculations access periods (IAPs) of 48 h at 25^{o}C, until 37 days after the beginning of the AAP. Inoculated test plants were kept in a vector-proof screened greenhouse. To assess successful inoculations, TaqMan® Real-time PCR detection assays with LAS-specific primers were performed on DNA extracts from test plants 10 months later. First transmission events were observed after 11 and 13 days from beginning of the AAP by 1st instar nymphs and adults, respectively. All nymphs inoculated the pathogen for the first time between 11 to 19 days. In contrast, first transmission events by adults were observed at more variables times, at 13, 15, 29, 31 and 33 days from beginning of the AAP. The results indicate that the mean latent period of LAS in *D. citri* is approximately 2 weeks at 25℃, varying with the ins ect development stage during acquisition.

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AN APHID VIRUS WITH A PLANT VIRUS-DERIVED COAT PROTEIN

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The soybean aphid, *Aphis glycines* Matsumura, is the most important insect pest of soybean in North America. Management of the soybean aphid has cost an estimated $1.6 billion over the last 10 years. Our goal is to identify viruses of the soybean aphid which have potential for use in soybean aphid management. The soybean aphid transcriptome was sequenced using Illumina/Solexa short read sequencing and the data screened for viral sequences. A new insect virus, *Aphis glycines* virus (AGV), was discovered. AGV is estimated to have a 5 kb single stranded RNA (ssRNA) genome and to form a 30 nm particle. The RNA-dependent RNA polymerase (RdRp) of this virus is closely related to that of *Euprosterna elaeasa* virus (EeV: Tetraviridae) and *Drosophila* A virus (DAV: unclassified). However, based on structural predictions, the AGV coat protein (CP) is more similar to the CP of plant viruses (*Necroviruses* and *Sobemoviruses*). Notably, a potential CP-readthrough protein (CP-RTP) is predicted from the AGV genome sequence. The AGV RTP has a polyproline track in the N-terminus of the RTP which is structurally similar to that of the *Luteoviruses.* Analysis of the RTP suggests that RTP may be involved in penetration of the host cell. AGV appears to have a 100% vertical transmission rate and has also been detected by RT-PCR in laboratory colonies of two other aphid species, the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus) and the green peach aphid, *Myzus persicae* (Sulzer). AGV represents a new group of insect viruses which appears to infect multiple aphid species.

ACQUISITION AND INOCULATION OF *TOMATO YELLOW LEAF CURL VIRUS* FROM RESISTANT GENOTYPES BY *BEMISIA TABACI*: RESISTANT GENOTYPES ARE RESERVOIRS OF THE VECTOR AND THE VIRUS

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Tomato yellow leaf curl virus (Family *Geminiviridae*, Genus *Begomovirus*) is transmitted by sweet potato whitefly, *Bemisia tabaci* (Gennadius). One of the main management options includes planting of *Tomato yellow leaf curl virus* (TYLCV) resistant genotypes. Resistant genotypes possess a semi-dominant gene (*Ty-1*) that confers resistance against TYLCV. However, they do not possess desirable horticultural characteristics and are also not available for all tomato fruit types. As a consequence, resistant and susceptible genotypes are often planted in close proximity. Resistant genotypes also do not provide any resistance against whiteflies. Field studies were conducted in 2009 and 2010 to evaluate the effect of resistant genotypes on TYLCV incidence and whitefly populations. Visual observations indicated that there was no TYLCV infection in resistant genotypes, the infection rates ranged from 45 to 85% in susceptible cultivars. However, further testing with PCR using degenerate *Begomovirus* primers followed by sequencing indicated that the resistant cultivars were actually infected with TYLCV, but were symptomless. Greenhouse inoculations using viruliferous whiteflies followed by PCR testing indicated that the resistant genotypes were infected up to 100% without exhibiting symptoms. Acquisition ability of whiteflies was tested on various genotypes. Results indicated that resistant genotypes differentially affected acquisition. Whitefly acquisition rates were lower when they fed on resistant genotypes than on susceptible genotypes. Nevertheless, this difference did not affect TYLCV inoculation from resistant genotypes to susceptible genotypes. Whitefly population densities in resistant and susceptible genotypes were similar. Results emphasize that resistant genotypes can potentially serve as inoculum sources for TYLCV and may influence its epidemiology.

STRAIN SPECIFITY AND SIMULTANEOUS TRANSMISSION OF CLOSELY-RELATED STRAINS OF A *POTYVIRUS* BY GREEN PEACH APHID, *MYZUS PERSICAE* (SULZER)

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Potato virus Y (PVY), (Family *Potyviridae*; Genus *Potyvirus*) is non-persistently transmitted by aphids. PVY severely affects potato production in the United States and worldwide. Single and mixed infections of PVY strains, namely PVY^{O}, PVY^{NTN}, and $PVY^{N:O}$ are a common occurrence in potato systems. However, information available on the ability of aphids to simultaneously transmit multiple PVY strains, specificity associated with simultaneous transmission of multiple strains, and factors affecting the same are limited. Aphid-mediated transmission experiments were conducted to initially evaluate the ability of individual aphids to transmit multiple strains using an indicator host. Preliminary results revealed that aphids can transmit at least two viral strains simultaneously. Subsequently, aphid-mediated transmission of three dual-strain combinations was tested using potato plantlets. Individual aphids transmitted two viral strains simultaneously for all three dual-strain combinations. In all aphid-mediated dual-strain infections involving PVY^{NTN}, the rate of PVY^{NTN} infection was higher than the second strain and the dual infection rate, indicating that there was specificity associated with transmission of PVY strains. Results of aphid-mediated transmission experiments were compared with results obtained through mechanical transmission. In general, PVY infection rates achieved by aphid-mediated transmission were lower than the rates achieved through mechanical transmission. Unlike aphid-mediated transmission, no strain or combination was eliminated through mechanical transmission. These results suggest that there may be interference associated with aphid transmission of closely-related PVY strains. Perhaps, the observed specificity and/or interference may explain the spike in the incidence of PVY^{NTN} and other necrotic strains in recent years.

A PHYTOPLASMA EFFECTOR TARGETS SPECIFIC PLANT TRANSCRIPTION FACTORS TO PROMOTE PROGENY PRODUCTION OF PHYTOPLASMA LEAFHOPPER VECTORS

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Phytoplasmas are insect-transmitted plant pathogenic bacteria that can induce dramatic changes in plant morphology and alter the fitness and behaviour of their insect vectors. We found that *Arabidopsis thaliana* infected with Aster Yellows phytoplasma strain Witches' Broom (AY-WB) produces many axillary stems (witches' broom symptoms). Furthermore, the generalist leafhopper *Macrosteles quadrilineatus*, which vectors AY-WB, and the maize specialist leafhopper *Dalbulus maidis*, which normally does not use Arabidopsis as a host plant, produced more progeny on AY-WB-infected Arabidopsis. We hypothesized that AY-WB phytoplasma produce virulent proteins (effectors) that modulate specific host targets leading to the changes in plant morphology and insect vector fitness. Previously, we sequenced and mined the genome of AY-WB and identified 56 candidate effectors. The Arabidopsis lines that express one of the effectors, SAP11, produced curly leaves and many stems. Furthermore, *M. quadrilineatus* produced more nymphs on SAP11 expression lines. We revealed that SAP11 binds and destabilizes Arabidopsis *CINCINNATA* (*CIN*)-related TCP transcription factors, which control plant development and promote the expression of lipoxygenase (*LOX*) genes, which in turn are required for jasmonate (JA) synthesis. *LOX* expression and JA production were reduced in the SAP11 expression lines. Furthermore, *M. quadrilineatus* produced more offspring on Arabidopsis JA synthesis and response mutants. Thus, SAP11 suppresses the JA mediated defense response to *M. quadrilineatus* by destabilizing TCPs leading to an increased number of the insect. As AY-WB relies on insect vectors for transmission, we hypothesize that SAP11 promotes AY-WB dispersal in nature by increasing *M. quadrilineatus* numbers. Unlike *M. quadrilineatus, D. maidis* did not increase its fecundity or survival rate on SAP11 expressing lines, indicating that in addition to SAP11 other AY-WB effectors may modulate Arabidopsis resistance to the non-host leafhopper, *D. maidis.*

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ACQUISITION OF 16SrIX, HLB ASSOCIATED PHYTOPLASMA, BY *SCAPHYTOPIUS MARGINELINEATUS* (HEMIPTERA: CICADELLIDAE) FROM *CROTALARIA JUNCEAE*

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Huanglongbing (HLB) is a severe disease of citrus associated with *Candidatus* Liberibacter spp. (liberibacter). Recently, phytoplasmas from group 16SrIX and from group 16SrI were also associated with HLB, independently from liberibacter. As of today, we have five bacteria associated with HLB as causative agents: *Ca*. Liberibacter asiaticus, *Ca*. Liberibacter africanus, *Ca*. Liberibacter americanus, group 16SrIX phytoplasma (Pigeon pea witches'-broom group) and group 16SrI phytoplasma (*Ca*. phytoplasma asteris group). Group IX phytoplasmas are associated with a diverse range of disease in different crops and are found associated with leguminous plant in some countries from Americas. Due to the association of group IX phytoplasma with HLB in Brazil, we have searched leguminous plants for occurrence of this phytoplasma. We found *Crotalaria junceae* displaying witches'-broom harboring HLB phytoplasma. The recent discovery of *Scaphytopius marginelineatus* as vector of HLB associated group 16SrIX phytoplasma, led us to assess the acquisition and transmission of this phytoplasma from crotalaria to citrus plants. Crotalaria plants with witches' –broom symptoms were collected in Potirendaba (SP), transferred to pots and kept in a greenhouse in Araraquara (SP). These plants were positive for HLB phytoplasma as determined by PCR and sequencing of PCR products. Adults from S. *marginelineatus* (250) were caged in crotalaria plants for 14 days and were kept for additional 7 days in latency period in *Sida rhombifolia*. After an inoculation period of 5 days in young citrus plants (20 per plant), leafhoppers were collected and groups of 5 individuals were employed for phytoplasma detection. On average, detection of HLB phytoplasma was positive in 80% of leafhopper samples. Citrus plants are under evaluation for HLB symptom development. Additional assays are necessary to prove transmission while no symptom is observed. This results show efficient acquisition of HLB 16SrIX phytoplasma from witches'-broom crotalaria plants by *S. marginelineatus*.

THE EFFECTS OF A VIRAL SILENCING SUPPRESSOR PROTEIN ON PLANT-APHID INTERACTIONS

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Infection with cucumber mosaic virus strain Fny (Fny-CMV) induced resistance to aphids (*Myzus persicae*) in *Arabidopsis thaliana* ecotype Col-0. Electrical penetration graph analysis of aphid feeding behavior and microbalance measurements showed that aphids fed less and grew poorly on infected plants, leading to the production of smaller colonies. Experiments with the Fny-CMV∆2b deletion mutant and *Fny2b*-transgenic plants showed that the CMV 2b RNA silencing suppressor protein induces aphid resistance. Consistent with this, glucosinolate accumulation increased in plants infected with Fny-CMV but not Fny-CMV∆2b. Fny-CMV infection caused dramatic changes in the transcriptome typically associated with salicylic acid (SA)-dependent defense responses (most likely due to induction of SA accumulation by the virus) while most jasmonic acid-regulated genes were unaffected. Nevertheless, experiments with defensive signaling mutants showed that neither hormone is critical for aphid resistance induction by Fny-CMV. In Arabidopsis Col-0, the Fny2b protein, but not the LS-CMV strain 2b protein, strongly inhibits microRNA-mediated turnover of host mRNA. Aphid resistance was not triggered in LS-CMV-infected or *LS2b*-transgenic plants, which suggests an important role for the microRNA pathway in regulating aphid resistance. The results in Arabidopsis contrasted with those from tobacco (*Nicotiana tabacum*) where Fny-CMV induced susceptibility to aphids and Fny-CMV∆2b induced a strong nicotine-independent resistance that caused increased mortality among these insects. The potential epidemiological implications of host-specific and CMV strain-specific effects on plant-aphid interactions will be discussed.

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