ELSEVIER

Abstracts / Comparative Biochemistry and Physiology Part A 141 (2005) S225 – S236

CBP

www.elsevier.com/locate/cbpa

Society for Experimental Biology Annual Main Meeting

11th–15th July 2005, Universitat Autonoma de Barcelona, Barcelona, Spain

P2–PHLOEM-INSECT INTERACTIONS

Organised by Jeremy Pritchard (University of Birmingham)

P2.1
EPG monitoring of action potential effects points to salivation as a weapon against sieve plate plugging by phloem proteins

A.J.E. van Bel and T. Will, (Plant Cell Biology Research Group, Institute of General Botany, Justus-Liebig-Universität, Senckenbergstrasse 17, D-35390 Giessen, Germany; Torsten.Will@bot1.bio.uni-giessen.de, Aart.v.Bel@bot1.bio.uni-giessen.de)

Only in sieve tubes of Fabaceae do Ca^{2+}-dependent contractile protein bodies (forisomes) occur. Burning *Vicia faba* leaf tips trigger an action potential along the sieve tube, which concurs with temporary calcium influx/efflux and corresponding dispersion/contraction of the forisome. Temporary sieve plate plugging by the dispersed forisome provokes a EPG-recorded E2>E1 transition of *Megoura viciae* feeding on sieve tubes. This change is interpreted as an attempt to remove plugging by salivation and to restore food supply. Similar behavioural reactions were observed for *Acyrtosiphon pisum* and *Aphis faba*. In plant species with other types of protein plugging such as *Brassica napus* (with *Myzus persicae*) and *Hordeum vulgare* (with *Schizaphis graminum*), aphid reacted to leaf tip burning by strongly increased E1 salivation. The latter combination allowed us to measure propagation speed and strength of the action potential by monitoring aphid behaviour at various distances from the leaf tip. Given the involvement of calcium in sieve plate plugging, aphids will be trying to limit intercellular calcium in sieve tubes by physical and chemical means. Stylet anatomy and mode of stylet insertion restrict calcium influx. Furthermore, intense salivation (E1) during sieve tube puncture and in response to sieve plate plugging suggests that saliva substances act as calcium chelators. As in vivo confocal microscopic methods fell short to tackle this question, we adopted an in vitro method in which the components of the aphid–plant interaction (saliva and phloem proteins), involved in sieve plate (un)plugging, are enabled to react.

Keywords: Action potentials, Aphid salivation, Sieve element calcium, EPG, Sieve element proteins

P2.2
Use of functional genomics in *Arabidopsis* to investigate the role of plant amino acid transporters in determining the performance of phloem-feeding aphid *Myzus persicae*. The use of *Arabidopsis* genetic resources to investigate plant–aphid interactions

S. Gattolin, E. Hunt, J. Bale, H.J. Newbury and J. Pritchard, (School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK)

Phloem amino acid concentrations are central to aphid performance, but the effect of a suboptimal diet is difficult to predict due to various compensatory responses that the insect can employ. The concentration of amino acids in the phloem can be altered environmentally; however the effect on phloem composition can be buffered by the plant. Our approach is to modify phloem sap with knockout mutations of defined plant amino acid transporter genes to investigate the relationship between the aphid *Myzus persicae* and a plant host, *Arabidopsis thaliana*. *Arabidopsis* amino acid transporters can be divided into a number of families and subgroups. We are currently focussing on ANT1, an aromatic and neutral amino acid transporter, and AAP6, a member of the broad-spectrum AAP family. We showed a significant increase in total phloem amino acid concentration in the *Arabidopsis* mutant *ant1* and a decrease in mutant *aap6* compared with the wild type. Preliminary data show that *M. persicae* feeding on the *aap6* mutant has a significantly decreased reproductive performance, and the use of the EPG technique with aphids feeding on *ant1* revealed that the time spent tapped into the phloem was reduced by over 60% in comparison to wild-type plants. Thus the absence of a single gene in *Arabidopsis* can affect either aphid feeding behaviour or reproductive performance. To further our study of the effect of phloem amino acids on plant–aphid interactions, we aim to obtain and study more AAP mutants and to cross pairs of mutants showing variations in amino acid composition, to produce further extremes of phloem composition.

doi:10.1016/j.cbpb.2005.05.018

P2.3
Plant nutritional quality and the mid-summer aphid population crash: the role of phloem amino acid composition

A.J. Karley[a,*], K.V. Pescod[a], J. Howard[b], W.E. Parker[b], J.W. Pitchford[a] and A.E. Douglas[a] ([a]University of York, Department of Biology, UK; [b]ADAS Wolverhampton, UK; * Current address: Scottish Crop Research Institute, Dundee, UK)

This study addresses the significance of phloem sap nutrient composition as a determinant of both the performance of phloem-feeding aphids and the dynamics of aphid populations. In Northern Europe, the populations of many aphid species utilising herbaceous plants, including crops, increase exponentially in early summer but 'disappear' from the plants, usually over a time-scale of a few days, in July. In our experimental system, potato (*Solanum tuberosum*) crops in the UK, aphid numbers decline around the time that plants divert much of their dry matter into tuber filling, indicating that changes in plant nutrient partitioning might play a role in the population crash. The hypothesis that developmental variation in phloem nutritional quality contributes to the mid-summer population crash has been investigated by a combination of laboratory and field-scale experiments. Analysis of phloem samples obtained by EDTA-facilitated exudation and by aphid stylectomy has revealed consistent changes in the relative abundance of the dominant non-essential amino acids in the phloem sap as the plants mature. Experimental manipulation of amino acid composition of aphid diets suggests that these seasonal changes in plant nutrients can have a small but significant impact on aphid growth and fecundity. A modelling approach based on the excitable media paradigm has been developed that allows examination of the relative contribution of changes in phloem nutritional quality and other factors controlling aphid population dynamics.

P2.4
Gene expression pattern in resistant and susceptible apple cultivars as a response to feeding of rosy apple aphids, *Dysaphis plantaginea* (Passerini)

T. Qubbaj, A. Reineke and C.P.W. Zebitz, (University of Hohenheim, Institute of Phytomedicine, D-70593 Stuttgart, Germany; e-mail: qubbaj@uni-hohenheim.de)

Understanding the complex transcriptional changes occurring after insect attack in plants is becoming increasingly important for the global study of plant–insect interactions and thus for an efficient management of insect pests.
In the present study, a novel RNA fingerprinting technique, cDNA-amplified fragment length polymorphism (cDNA-AFLP), has been applied to elucidate the molecular and biochemical mechanisms involved in the resistance of apple trees, *Malus domestica* L., against its primary aphid pest, the rosy apple aphid *Dysaphis plantaginea* (Passerini), which is considered a serious economic pest infesting apple. Gene expression in both resistant and susceptible apple cultivars after infestation with rosy apple aphids, as well as the plant response to *D. plantaginea* attack in a time series, was investigated.
Fragments differentially expressed in both cultivars were cloned and sequenced, and their pattern of gene expression was subsequently verified by Northern blots. Sequence comparisons of differentially expressed fragments to databank entries revealed homologies to already known genes, most of them isolated from *Arabidopsis thaliana* L. Genes that showed a homology to apple cDNA fragments of the cv. "Florina" included an RNase-L inhibitor-like protein, a pectin-acetyl esterase, an inositol-phosphatase-like protein, a precursor of the large chain of the ribulose-1,5-biphosphate-carboxylase, and defence-related genes such as a vacuolar H(+)-ATPase subunit-like protein, an ADP-ribosylating enzyme, and chitinase. The results are discussed in relation to a putative role of these genes in conferring aphid resistance in apple trees, and in understanding the mechanisms of defence in apple.
Keywords: *Dysaphis plantaginea*, *Malus domestica*, Resistance, Gene expression

P2.5
Phloem sap utilisation by aphids: symbiotic problems and solutions

A.E. Douglas, (University of York, UK; aed2@york.ac.uk)

The capacity to utilise plant phloem sap as sole diet has evolved multiple times in hemipteran insects, but no other animals. All phloem sap-feeding hemipterans possess symbiotic microorganisms widely accepted to provide the insect with nutrients deficient in phloem sap. Most research focused on the aphid–*Buchnera* symbiosis. Building on physiological and genomic evidence that *Buchnera* provide aphids with essential amino acids, I will address: (a) the impact of plant traits, especially phloem sap nutrient composition, on symbiosis function; and (b) how variation in the microbial symbiosis affects the capacity of aphids to utilise plant sap of varying compositions. My primary focus will be phloem amino acids and sugars. The priority for future research will be to integrate the increasing availability of molecular and genomic approaches to dissect symbiosis function with the nutritional physiology of the three-way interaction among the plant, insect, and symbiotic microorganisms.

P2.6
Global transcriptional responses of *Buchnera aphidicola* to aromatic amino acid limitation in the diet of its symbiotic partner, the phloem sap-feeding insect *Acyrthosiphon pisum*

F. Calevro[a,b], N. Reymond[a], N. Morin[a], Y. Rahbé[a], J. Bernillon[c], G. Febvay[a], C. Laugier[a,b], J.M. Fayard[a,b] and H. Charles[a,b], ([a]UMR 203 INRA/INSA de Lyon, Biologie Fonctionnelle, Insectes et Interactions, INSA, Bât. Louis Pasteur, 69621 Villeurbanne Cedex, France; [b]Biologie des Systèmes et Modélisation Cellulaire, France; [c]DTAMB, Université Claude Bernard Lyon I, Bât. G. Mendel, 69622 Villeurbanne Cedex, France; http://bsmc.insa-lyon.fr/)

All insect groups using plant sap as the principal or sole source of food depend, for growth and reproduction, on symbiotic micro-organisms and most aphids bear intracellular bacteria of the genus *Buchnera*. This association is obligate for both the aphid and

Buchnera. As for the other obligate host-associated bacteria, the nutritional role of the symbiont reflects the inadequacy of the insect food sources. *Buchnera* furnishes vitamins and essential amino acids that the aphid host can neither synthesize nor find on the phloem sap. Aphid demand for essential amino acids is variable, depending on phloem sap amino acid composition and content, and aphid developmental age or physiological conditions. We might expect that *Buchnera* is able to vary its amino acid delivery according to the aphid demand. However, genomic and first transcriptomic data contribute little to the understanding of the integration of *Buchnera* into the nutritional physiology of the aphid.

The aim of this work was to analyze the transcriptomic response of *Buchnera* when the aphid host is submitted to a depletion of two amino acids essential to its development: tyrosine and phenylalanine. To do this, we generated a 35-mer oligonucleotide-based microarray covering the whole genome of the primary symbiont of the aphid *Acyrthosiphon pisum*. Our analysis reveals that *Buchnera* is able to respond at the transcriptome level to this nutritional stress. Genes sharing significantly altered expression in depleted diet principally include tRNA and genes involved in tRNA biosynthesis and metabolism, amino acid biosynthesis, and potential transport-related systems.

P2.7
Endosymbiotic fauna of different host-associated populations of *Aphis gossypii* in Australia—is there any interaction between host plant use and microbiota composition?

A. Najar, G.H. Walter and E. McGraw, (Department of Zoology and Entomology, The University of Queensland, Australia; a.najarrodriguez@uq.edu.au)

Worldwide, *Aphis gossypii* populations consist of a complex of anholocyclic lines with many apparently having specific host plant associations. This wide range of host plant associations and the differential host-associated behaviour of *A. gossypii* have been interpreted as an indication that the current definition of this species may include a complex of cryptic species. However, none of the studies carried out to test this hypothesis has yielded supportive results. Endosymbiotic bacteria in aphids have been found to influence ecologically important traits, including host plant use. To date, no information is available on any such endosymbiotic associations in *A. gossypii* in any part of its host range. This paper aims at investigating the relationships between these aphid/endosymbiotic associations across populations of *A. gossypii*. A molecular approach was taken to characterise the endosymbiont community of the different aphid populations. Using a combination of denaturing gradient gel electrophoresis and sequencing techniques, we screened for the presence of the PASS (pea aphid secondary symbionts), PAUS (pea aphic U-type symbiont), PABS (pea aphid *Bemisia*-type symbiont), and PAR (pea aphid *Rickettsia*) as well as any previously unidentified species. Electron microscopy was used to determine the exact position of the endosymbionts within the aphid body. Finally, the effects of the elimination and artificial transmission of endosymbionts on aphid performance across hosts were assessed.

Keywords: Aphis gossypii, Host plant interactions, Endosymbiotic fauna

P2.8
Functional diversity in *Capsella bursa-pastoris*—the impact on insect herbivores

A.J. Karley, C. Hawes, P.P.M. Iannetta and G.R. Squire, (Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK; akarle@scri.sari.ac.uk)

Detecting the level and functional significance of diversity in arable systems is of primary importance in agro-ecology. Within-species variation in the physiology and life history traits of weed species could be an important component of ecological diversity, particularly in intensively managed arable systems. Intra-specific variability has been rigorously exploited in the development of new crop varieties, but non-crop species have received little attention. Our research explores the importance of intra-specific diversity in weeds for other trophic levels, using *Capsella bursa-pastoris* as a model. *C. bursa-pastoris*, or Shepherd's-purse, is a common and widespread weed of arable systems in the UK and throughout northern temperate regions. Within-species variation in a number of traits has led to classification schemes based on leaf morphology[1], life history traits[2], and molecular markers[3]. Parent lines collected from arable sites across the UK have been characterised ex situ and show wide variation in a number of phenotypic traits relating to plant growth, appearance, and resource allocation, including phloem amino acid composition.

The potential impact of these phenotypic differences on insect trophic groups is being explored through glasshouse studies of aphid performance on a range of *C. bursa-pastoris* ecotypes, and field experiments on the insect communities associated with two extreme ecotypes. Implications of these studies for the relative importance of within-species variation in the diversity and functioning of arable systems are discussed.

Keywords: *Capsella bursa-pastoris*, Functional diversity, Trophic group, Phloem composition, Aphid

[1]Shull (1909). *Carnegie Institution Publications* 112, 1–57.
[2]Hurka and Neuffer (1997). *Plant Systematics and Evolution* 206, 295–316.
[3]Linde et al. (2001). *Annals of Botany* 87, 91–99.

P2.9
Natural products in plant–insect interactions: the myrosinase–glucosinolate system in *Arabidopsis* and the specialist aphid *Brevicoryne brassicae*

John T. Rossiter (Imperial College, Wyc Campus, UK; j.rossiter@imperial.ac.uk)

Secondary metabolites in plants are thought to function as deterrents to nonadapted herbivores, while adapted herbivores have developed mechanisms of detoxification or even utilize such compounds in their own defence. All cruciferous plants contain glucosinolates, and recent developments in *Arabidopsis* genomics have enabled the complex secondary metabolite biosynthetic pathway to be elucidated and provide an ideal model to study plant–insect interactions. Glucosinolates and their degradation products are involved in plant–insect interactions and we have previously shown that the cabbage aphid, *Brevicoryne brassicae*, has evolved to a defence system that mirrors that of its host plant. Like the host plant, the cabbage aphid contains both myrosinase

and glucosinolates. The location of myrosinase in non-flight skeletal muscle of the aphid signifies an important adaptive evolutionary defence mechanism that mimics the biochemistry of the host plant. The cellular mechanisms for the synthesis of aphid myrosinase, together with the role of the enzyme in multitrophic interactions, will be discussed together with the organisation of the myrosinase–*Arabidopsis* system in *Arabidopsis.*

P2.10
Salivary secretion activities during plant penetration by aphids

W. Fred Tjallingii, (Wageningen University, Laboratory of Entomology, The Netherlands; freddy.tjallingii@wur.nl)

Phloem feeding implies overcoming a number of phloem-located plant properties and reactions. The most important hurdles are the phloem wound responses, such as coagulating proteins in the sieve element and in the stylet's capillary food canal of the insect. It seems likely that in order to prevent protein clogging inside a sieve element, the ejection of watery saliva plays an important role. Phloem salivation always precedes phloem feeding as shown in electrical penetration graphs (EPGs) as waveform E1 preceding waveform E2. Concurrent to (passive) phloem sap feeding (E2), on the other hand, watery saliva is added regularly to the imbibed sap, most likely to prevent clogging of phloem proteins inside the capillary food canal. Whatever the biochemical mode of action might be in the inhibition of protein coagulation, it seems possible that not all aphids are able to prevent clogging in all plant species, which implies that only certain plants species are suitable as a host plant for certain species of phloem feeders. Those plants that are not suitable can be considered as resistant to these insects, consequently. Evidence will be presented for such phloem-located resistance to aphids, based on EPG signals.

P2.11
A proteomics approach to identify key components of aphid saliva

J. Carolan and T. Wilkinson, (Department of Zoology, University College Dublin; jim.carolan@ucd.ie, tom.wilkinson@ucd.ie)

Phloem-feeding insects can puncture and feed from sieve elements for long periods apparently without initiating plant wound responses. Salivary secretions from the insect are thought to play a critical role in preventing the sealing of the sieve elements and/or insect mouthparts during feeding. We have adopted a comparative two-dimensional gel electrophoresis approach to investigate the protein components of saliva that allow aphids to ingest phloem for long periods. A key strategy in this research is the analysis of saliva from symbiont-free aphids. These insects display abnormal salivation behaviour during feeding, which may be indicative of changes in the protein composition of the saliva.

P2.12
Priming phloem-based resistance to aphids

G. Powell[a], S. Hodge[a] and G.A. Thompson[b], ([a]Division of Biology, Imperial College London, Wye Campus, Ashford TN25 5AH, UK; g.powell@imperial.ac.uk; [b]Department of Applied Science, University of Arkansas at Little Rock, Arkansas 72204-1099, USA)

β-Aminobutyric acid (BABA) is a non-protein amino acid that is an effective inducer of resistance against a variety of plant pathogens. However, examples of BABA-induced resistance against phytophagous insects have not been reported. We have demonstrated that BABA applied as a soil drench to legumes reduces the performance of the pea aphid (*Acyrthosiphon pisum*). On tic bean (*Vicia faba*), BABA caused a dose-related reduction in the mean relative growth rate (MRGR) of individual aphids and also their intrinsic rate of population increase (r_m). The reduction in aphid performance may be linked with BABA-induced phytotoxic stress or direct toxicity to aphids, but a series of experiments has provided no evidence for these two possibilities. Our results instead point to a BABA-induced aphid resistance mechanism. Electrical penetration graph (EPG) recordings on *V. faba* indicate that sieve element defences may be enhanced on BABA-treated plants, leading to disrupted phloem sap ingestion by *A. pisum*. Since aphid resistance genes often operate via phloem-specific mechanisms, it is possible that R-gene-mediated resistance and BABA-induced resistance share common features. Current EPG experiments are therefore investigating interactions/commonalities between these two types of aphid resistance.

Keywords: Induced resistance, Defence activator

P2.13
Sieve elements and whitefly resistance in alfalfa

G.P. Walker and Y.X. Jiang, (Department of Entomology, University of California, Riverside, USA; walker@citrus.ucr.edu)

Alfalfa genotypes highly resistant to the silverleaf whitefly, *Bemisia argentifolii*, have been identified, and resistance is expressed primarily as very-high first instar mortality. Stylet penetration by first instar whiteflies and their rates of honeydew production were compared between resistant and susceptible alfalfa genotypes. Both histological and electrical penetration graph (EPG) techniques indicate that first instar whiteflies are able to locate and penetrate sieve elements with equal success on resistant and susceptible alfalfa genotypes. However, after penetration of a sieve element, ingestion (as measured indirectly by honeydew excretion) is greatly reduced on resistant genotypes compared to susceptible genotypes. Thus, the mechanism(s) of resistance appears to reside in the sieve elements. Subsequent experiments were conducted to determine if the mechanism(s) of resistance is due to sieve element blockage by callose or P-protein, and to compare the details of stylet behavior within sieve elements between resistant and susceptible alfalfa genotypes.

Keywords: Crop resistance, Resistance mechanisms, Aleyrodidae, Medicago, Phloem

P2.14
Comparative structural damage caused to phloem by aphids feeding on wheat and barley cultivars

C.E.J. Botha[a], L.-A. de Wet[a], H. van Schalkwyk[a], S.A. Saheed[a], L. Liu[a] and L. Jonsson[b], ([a]Department of Botany Rhodes University

Grahamstown South Africa; [b]The Department of Plant Biology Södertörn University, Sweden; T.Botha@ru.ac.za)

Withdrawn

P2.14
Local adaptation of *Myzus persicae* to lupin feeding in Western Australia involves improved tolerance of phloem-transported alkaloid

Y.J. Cardoza, S. Seah, J. Reidy-Crofts and O.R. Edwards, (CSIRO Entomology, Private Bag 5, Wembley WA 6913, Australia; Owain.Edwards@csiro.au)

We compared the performance on narrow-leafed lupin (*Lupinus angustifolius* L.) of a representative lupin-feeding Western Australia (WA) clone of the generalist aphid *Myzus persicae* (Sulzer) to nine clones collected from Australian locations where narrow-leafed lupins rarely occur. The WA clone showed significantly higher mean relative growth rate (MRGR) and colonisation ability on three lupin varieties, indicating that successful lupin feeding is not a characteristic of the species. High-pressure liquid chromatography analysis of phloem collected from the different varieties yielded differences in the quantities of two alkaloids. The WA lupin clone showed significantly improved performance compared to two other clones on artificial diet amended with one of these alkaloids, lupanine. These results suggest that the WA clone of *M. persicae* has become locally adapted to feed successfully on narrow-leafed lupin, and that this adaptation may involve improved tolerance of lupanine in their diet. Differential display methods are being used to identify genes differentially expressed between lupin-feeding and non-lupin-feeding clones. Candidate genes that correlate to lupin feeding success will be identified by comparison to EST databases currently being developed for this aphid species.

Keywords: Aphids, Insect clones, Plant resistance, Host adaptation, Alkaloids, Lupins

P2.15
The novel insect-defense cysteine protease, Mir1-CP, is localized in the phloem of maize leaves

D.S. Luthe[a], L. Lopez[a], A. Camas[a], R. Shivaji[a], and W.P. Williams[b], ([a]Department of Biochemistry and Molecular Biology, Mississippi State University; [b]Corn Host Plant Resistance Laboratory, Mississippi State University; dsluthe@ra.msstate.edu)

Fall armyworm (*Spodoptera frugiperda*, FAW) is a major insect pest of corn (*Zea mays* L.). Using the inbred line Mp708, with resistance to FAW larval feeding, we previously reported that a unique 33-kDa cysteine proteinase (Mir1-CP) plays a role in conferring resistance to insect attack. Mir1-CP (maize insect resistance 1-cysteine protease) is a papain-like cysteine protease that accumulates in the whorls of insect-resistant maize genotypes in response to wounding, senescence, and feeding by lepidopteran larvae. We have shown that growth of FAW larvae that fed on transgenic callus ectopically expressing Mir1-CP was retarded by approximately 80% and that Mir1-CP damages the insects' peritrophic matrix, impairing nutrient utilization. The objective of this study was to investigate the Mir1-CP transcript and protein localization in the whorl during wounding and insect defense. By using in situ hybridization, silver enhancement, and immunogold immunolocalization, we found that Mir1-CP is located predominantly in the phloem of minor and intermediate veins of the leaf after FAW larval feeding. After larval feeding, the protein increases in abundance and moves symplastically through plasmodesmata from the vascular parenchyma cells to the unique thick-walled sieve element, the bundle sheath, and the mesophyll cells. Furthermore, it appears that the protein is exported into the apoplastic space between the vascular parenchyma cell and the vessels of the minor veins. The transport of Mir1-CP to the phloem thick-walled sieve elements may be a new type of cell-to-cell communication between a vascular parenchyma cell and a sieve element, which enables the maize phloem thick-walled sieve element to play a role in herbivore defense.

Keywords: Cysteine protease, Herbivory, Phloem, Maize, Defense

P2.17
Phloem proteins as potential factors influencing plant–insect interactions

J. Kehr, (Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany; kehr@mpimp-golm.mpg.de)

A potential class of molecules in the phloem that can influence plant–insect interactions is comprised of proteins and peptides, whereof more than a hundred have been detected in phloem saps of different species. Meanwhile, the identification of some of the phloem sap polypeptides [1–4] allows first insights into their possible functions. The currently provided information, although far from being complete, already indicates that some of them could be involved in processes like signal transduction [5,6], wound responses [7], and defense [1,8], with impact not only restricted to the phloem. This talk will update and summarize the current knowledge about phloem sap proteins and highlight their potential impact on plant–insect interactions.

[1] Walz, C., Juenger, M., Schad, M., Kehr, J., *Plant J*., 2002, 31, 189–197.
[2] Walz, C., Giavalisco, P., Schad, M., Juenger, M. et al., *Phytochemistry*, 2004, 65, 1795–1804.
[3] Hayashi, H., Fukuda, A., Suzui, N., Fujimaki, S., *Aust. J. Plant Physiol.*, 2000, 489–496.
[4] Barnes, A., Bale, J., Constantinidou, C., Ashton, P. et al., *J. Exp. Bot.*, 2004, 55, 1473–1481.
[5] Pearce, G., *Science*, 1991, 253, 895–898.
[6] Nakamura, S., Hayashi, H., Mori, S., Chino, M., *Plant Cell Physiol*., 1995, 36, 19–27.
[7] Clark, A.M., Jacobsen, K.R., Bostwick, D.E., Dannenhoffer, J.M. et al., *Plant J.*, 1997, 12, 49–61.
[8] Christeller, J.T., Farley, P.C., Ramsay, R.J., Sullivan, P.A. et al., *Eur. J. Biochem.*, 1998, 254, 160–167.

P2.18
Molecular interactions between crop plants and phloem-feeding aphid

Yinghua Huang, (USDA-ARS, Plant Science Research Laboratory, 1301 North Western Road, Stillwater, OK 74075, USA; e-mail: yinghua.huang@ars.usda.gov)

Greenbug (*Schiaphis graminum*), a phloem-feeding insect, is a dominant insect pest that attacks several cereal crops including sorghum, barley, and wheat. Using greenbug and sorghum as the

model, our current studies focus on molecular plant–aphid interactions. Plants express an array of defenses in response to pest attack through a dramatic functional adjustment and reprogramming of gene expression. In this study, genomewide microarrays and functional genomics were used to comprehensively examine gene expression during plant defense responses that were triggered by greenbug feeding. Over 8200 unique sorghum cDNA clones selected by suppression subtractive hybridization were arrayed on glass slides using robotic printing, and then hybridized with fluorescent-labeled probes prepared with RNAs from resistant and susceptible lines as well as the RNAs from plants before and during infestation. In this way, a large number of differentially expressed genes were identified by cDNA microarrays. Their differential expression patterns were confirmed by two validation technologies: Northern blotting and real-time PCR. Comparison of the transcriptional profiles in the parallel assays revealed distinct expression patterns among these assays, reflecting that the resistant and susceptible sorghum plants differentially responded to infestation by virulent greenbugs at the molecular level. Information generated in the studies opened doors to new levels of understanding of plant–pest interactions. Integration of gene expression data into biochemical or metabolic characteristics allows a logical interpretation of the cross-talk between different defense pathways governing defense gene expression and of the diversity of proteins involved in the resistance and susceptibility in this crop.
Keywords: Defense pathways, DNA microarray, Functional genomics, Greenbug, Sorghum

P2.19
Genetics of phloem–aphid interactions in *Medicago truncatula*

J. Klingler[a,b], R. Creasy[c], L.-L. Gao[a,b], R.M. Nair[d], H. Spafford Jacob[c], O.R. Edwards[a] and K.B. Singh[b], ([a]CSIRO Entomology and [b]CSIRO Plant Industry, Private Bag 5, Wembley, WA 6913, Australia; [c]School of Animal Biology, University of Western Australia, Crawley, WA 6009, Australia; [d]South Australian Research and Development Institute (SARDI), GPO Box 397, Adelaide, SA 5001, Australia; e-mail: kli026@csiro.au)

Medicago truncatula (barrel medic) is grown as forage in Australia, where plant breeders have identified aphid-resistant *M. truncatula* germplasm. The status of *M. truncatula* as a model legume provides an excellent system to study defence against aphids at physiological, biochemical, and molecular levels. Aphid resistance has been backcrossed into susceptible cultivars to create pairs of resistant and susceptible near-isogenic *M. truncatula* lines. We are focusing on these pair of lines to identify mechanisms of defence against *Acyrthosiphon kondoi* (bluegreen aphid) and *Therioaphis trifolii* f. *maculata* (spotted alfalfa aphid). Resistance to *A. kondoi* is phloem-specific and requires an intact plant, suggesting transport of a resistance factor(s) in the phloem. *T. trifolii* resistance blocks systemic vein chlorosis, a damage symptom normally caused by this aphid in susceptible hosts. This damage-resistance phenotype suggests an interaction between a resistance factor(s) in vascular tissue and a bioactive component(s) within *T. trifolii* saliva. These two aphid resistance traits are controlled by separate, but linked, dominant genes: *AKR* (*A. kondoi* resistance) and *TTR* (*T. trifolii* resistance). Each gene is tightly linked to members of the NBS-LRR gene family, a group that can encode resistance to a broad range of agricultural pests including microbial pathogens and phloem-feeding insects. Our results suggest that *AKR* and *TTR* may also be members of this family. We are currently testing for a possible interaction between these two genetic loci in mediating resistance against these two aphid species.
Keywords: *Acyrthosiphon kondoi*, *Therioaphis trifolii,* NBS-LRR

P2.20
Ascorbic acid in the plant phloem: implications for plant–insect interactions

R. Viola and R.D. Hancock, (Unit of Plant Biochemistry, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK; rhanco@scri.sari.ac.uk)

Phloem-feeding aphids are distributed throughout all global crop production centres where they can have negative impacts on both crop yield and quality. The negative impacts of aphid colonisation can either be direct (with severe infestations resulting in feeding damage) or indirect (by increasing the plants' susceptibility to opportunistic pathogens or by acting as vectors for viral diseases). Control of aphid pests is currently achieved through breeding for resistance to infestation, the use of natural and synthetic insecticides, and biological control with increasing emphasis being placed on integrated pest management. One problem encountered with all of these control methods is that their specificity is either narrow (specific genes) or broad (indiscriminate chemicals).
We present an overview of the role of L-ascorbic acid (AsA) in plant–aphid interactions with particular reference to aphid dietary requirements for this antioxidant. In addition, we discuss the role of phloem-derived AsA and AsA metabolising aphid saliva enzymes in the redox modulation of the activity of phloem-derived defensive compounds. We propose that manipulation of phloem AsA content could provide a further opportunity within an integrated pest management framework and demonstrate that phloem-derived AsA comes from multiple sources. Finally, we suggest mechanisms by which phloem AsA concentration could be manipulated to exclusively target phloem-feeding pests.
Keywords: Aphid, Nutrient, Crop protection, Biosynthesis, Transport

P2.21
Systemic xenobiotics for control of sucking pests

R. Lind and B. Cheek, (Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK; rob.lind@syngenta.com)

Sucking pests are rapidly coming to the forefront of pest importance in many cropping systems. This has been triggered by the effective control of Lepidopteran and Coleopteran pests by a growing range of new and existing active ingredients (AI's) and by the recent introduction of specific GMO solutions for their control. No GMO solutions currently exist for sucking pest suppression and the chemicals used for their control are limited in terms of new chemistry, principally the neonicotinoids. Insecticide resistance to all chemical classes used for the control of sucking pests is either established or becoming apparent, and discovery of new AIs for the future control of sucking pests is required.
Biokinetics, the study of uptake, distribution, metabolism, and excretion, is key for understanding the translocation of AI from

spray droplets or seed treatments to the active target site within the insect. In sucking pests, we know very little about some of these processes. Understanding drug biokinetics in both plants and our model system, the peach potato aphid *Myzus persicae* is a key component in the development of new AIs for sucking pest control. Analytical methodology, based on liquid chromatography mass spectrometry, has been applied to plant and aphid systems to understand how newly discovered insecticides are internalised by aphids from plant tissues, metabolised, and excreted. The output of these studies helps identify metabolic weak points or poor physical properties in a molecule, which focuses on future synthetic chemistry in a series.

Keywords: Aphid, Myzus persicae, Biokinetics, Metabolism, Liquid Chromatography Mass Spectrometry

P2.22
Mechanism of phloem-based resistance in melon (TGR-1551) to the aphid *Aphis gossypii*

E.I. Garzo[a], A. Fereres[b], M.L. Gómez-Guillamón[c] and W.F. Tjallingii[a], ([a]Laboratory of Entomology, Wageningen University, Binnenhaven 7, Wageningen, The Netherlands; gossypii98@yahoo.es; [b]Instituto de Ciencias Agrarias-Centro de Ciencias Medioambientales (CSIC), Serrano 115 dpdo, 28006 Madrid, Spain; [c]Estación Experimental 'La Mayora' (CSIC), Algarrobo-Costa, 29750 Málaga, Spain)

Ultrastructural representation of sieve element penetration by *Aphis gossypii* (Hemiptera: Aphididae) in resistant TGR-1551 and susceptible control melons (*Cucumis melo*) was evaluated. Previous electrical penetration graph (EPG) recordings of probing by *A. gossypii* on accession TGR-1551 showed a longer phloem salivation phase (E1 phase) (Garzo et al., 2002). The activation of a defence response such as P-protein clogging or callose formation is likely to occur, creating a mechanical barrier for passive phloem ingestion (E2) by the aphid. The aim is to compare the ultrastructure of sieve plates (SP) and sieve element (SE) contents with respect to wound response proteins in susceptible (Regal) and resistant (TGR-1551) melon. Aphid probing was EPG recorded until the aphid showed phloem activity (E1 or E1+E2). After 5 min of phloem activity, the aphid stylets were cut by stylectomy with RF microcautery. The leaf tissue containing the stylet stump was dissected and processed for transmission electron microscopy (TEM).

Garzo, E., Soria, C., Gomez-Guillamon, M.L. Fereres, A. 2002. Feeding behavior of *Aphis gossypii* resistant accessions of different melon genotypes (*Cucumis melo*). *Phytoparasitica* 30: 129–140.

Keywords: *Aphis gossypii*, Resistant melon accessions, Phloem resistance mechanism, Wound response, P-protein

P2.23
Divergent defensive pathways in tomato, and their effects on plant–aphid interactions

F.L. Goggin, (University of Arkansas, Fayetteville, AR, USA; fgoggin@uark.edu)

Feeding by the potato aphid, *Macrosiphum euphorbiae*, on tomato (*Lycopersicon esculentum*) upregulates the expression of marker genes associated with induction of salicylic acid and jasmonic acid. Salicylic acid (SA) is a plant signaling compound that plays a key role in systemic acquired resistance to pathogens. Jasmonic acid (JA) likewise regulates induced defenses against many insect herbivores, including caterpillars, thrips, and mites. A major goal of our work is to determine how SA and JA interact to influence the outcome of the plant–aphid interaction. Treating plants with exogenous JA or an SA analog induces systemic defenses that reduce aphid population growth, which suggests that both of these plant hormones may play a role in limiting aphid infestation. In some cases, however, SA and JA can have mutually inhibitory effects, and so simultaneous induction of these compounds could potentially attenuate plant defenses against aphids. Our laboratory is currently using tomato lines deficient in JA and/or SA signaling to evaluate the adaptive value of SA- and JA-mediated plant responses to aphids.

Keywords: Jasmonic acid, Induced resistance, Salicylic acid, Systemic acquired resistance

P2.24
Nature of aphid-transmitted polerovirus particles in the phloem of cucurbits; involvement of phloem components in aphid transmission efficiency of these viruses

B. Bencharki[a], C. Reinbold[a], M. Erdinger[a], L. Schaeffer[a], V. Ziegler-Graff[b] and V. Brault[a], ([a]INRA-Biologie des Interactions, Virus-Vecteur 28, rue de Herrlisheim 68021 Colmar, France; [b]IBMP-CNRS 12 rue du Gl Zimmer, 67084 Strasbourg, France; Brault@colmar.inra.fr)

Phloem tissue is a crucial compartment in poleroviruses infection. These viruses are restricted to phloem tissue and are strictly transmitted by aphids in a circulative and nonpropagative mode. In the plant, poleroviruses are believed to assemble in the cytoplasm of companion cells and to move to distal parts of the plant through the sieve elements as intact particles. *Polerovirus* particles are composed of two structural proteins: the major coat protein (CP) and a minor component called the readthrough protein (RT). Aphid transmission of virus is strictly dependent on the presence of RT protein in virions. RT is also required for efficient long-distance movement. We have designed experiments to identify the nature of the viral complex being transported in phloem sap and acquired by aphids.

The RT protein found in the virions after purification is a C-terminal truncated form of the complete protein detected in total extracts of infected plants. The significance of this cleavage is unknown. The presence of the full-length form of the RT protein in the phloem sap of polerovirus-infected cucumbers suggests that the entire protein is present on virions undergoing long-distance transport.

Preliminary experiments have also shown that addition of phloem sap to the purified suspension of virus being delivered to aphids can significantly increase transmission efficiency. Identification of phloem components able to bind virions and susceptible to affect aphid transmission efficiency is under investigation.

Keywords: Polerovirus, Aphid transmission, Phloem proteins, Cucurbits

P2.25
Transcriptomics in plant–insect interactions and the functional genomics of plant defense induction by phloem-feeding insects

Gary A. Thompson, (University of Arkansas at Little Rock)

Transcriptome-wide analyses of gene expression during plant–insect interactions provide a "big picture" context in which to integrate the many physiological processes that occur in both organisms before, during, and after herbivory. These analyses are being extended to reveal information about herbivory-related events that occur among multiple organisms at different trophic levels. In particular, the relationship between phloem-feeding insects and plants offers an intriguing example of a biotic interaction. This is due to the unique anatomy and physiology of the phloem, the unusual dietary composition of the phloem sap, the modifications that have evolved in phloem-feeding insects to utilize the phloem sap as a food source, and the influence of associated microorganisms. As a consequence, the perception, signal transduction, and responses by the plants are anticipated to be different from those of insects that use other modes of herbivory or plant pathogens. Remarkably, there exists a universality in plant responses that reveals common themes between different phloem-feeding insects and other plant–biotic interactions. As these data become available, it is increasingly important to discriminate among changes in gene expression that contribute directly or indirectly to an effective defense. Genetic resistance, most often found in crop plants, as well as model systems that do not have ready sources of genetic resistance are contributing to our understanding of the signaling, metabolic, and defense pathways that determine an effective plant defense against phloem-feeding insects. Specific examples of transcriptomewide analyses of plant responses to phloem-feeding insects will be discussed.

Keywords: Aphid molecular biology, Host preference, Plant-insect interaction, Sorghum

P2.26
Development of SSH libraries and cDNA microarrays: systems for gene expression profiling and functional genomics of greenbug

Yinghua Huang, (USDA-ARS, Plant Science Research Laboratory, 1301 North Western Road, Stillwater, OK 74075, USA; E-mail: yinghua.huang@ars.usda.gov)

The study of aphid–plant interactions can be greatly enhanced by the development of genomic tools for both an insect and its host plant. In this study, two subtractive cDNA libraries were constructed for greenbug using a PCR-based suppression subtractive hybridization (SSH) method. The two greenbug cDNA libraries were developed from two populations of mRNA derived from greenbugs feeding on a preferred host (referred as library 1) and a non-preferred host (library 2), respectively. Each cDNA population was enriched using the second cDNA population as the driver in cDNA subtraction. In addition, a normal unsubtracted cDNA library was also constructed to facilitate cloning of full-length cDNAs. Subsequently, high-throughput screening of the SSH cDNA libraries was preformed using DNA microarray analysis, in which a total of 2304 selected cDNA clones represent 1536 unique transcripts from library 1 and 768 from library 2. In this way, special groups of differentially expressed genes were identified relevant to the feeding tests. The results generated from this study demonstrate that SSH technique and cDNA microarray are valuable tools for functional genomics of aphids. This powerful SSH technique has been able to compare two mRNA populations and obtained cDNAs of special genes that are either overexpressed or exclusively expressed in one population compared with another. This set of greenbug genes is an important resource both for identifying genes and gene regulation networks with key roles in compatible and incompatible plant-greenbug interactions and for understanding the genetic mechanisms underlying the virulence of aphids and plant defense response.

P2.27
Expression profiling of aphid-induced responses in barley

G. Delp, K. Larsson, I. Zetterlund and L. Jonsson, (Södertörns Högskola, School of Life Sciences, SE-14152 Huddinge, Sweden; lisbeth.jonsson@sh.se)

Barley is host to the bird cherry-oat aphid (*Rhopalosiphum padi*), one of the main pests in spring-sown cereals. Different barley lines vary in their resistance to this aphid. Using specific antibodies, it has been shown that pathogenesis-related proteins, such as chitinase and β-1,3-glucanase, are induced.

To obtain a broad picture of changes in gene regulation upon aphid attack, subtracted cDNA libraries from infested and non-infested barley plants have been screened for differentially expressed clones. An *O*-methyltransferase (OMT; accession no. U54767) has been further investigated. The putative OMT has been expressed in *Escherichia coli* and purified. It acts on AMI and MAMI, intermediates in the biosynthesis of the secondary metabolite gramine. Thus it acts on different substrates than previously reported and is here proposed to be involved in the biosynthesis of defence chemicals.

Recently we initiated differential expression profiling using the barley1 GeneChip (Affymetrix) in DNA microarray experiments. Our aim is to compare the pattern in resistant varieties with that in susceptible ones in order to identify the genes involved in aphid resistance. Out of 21429 genes represented on the chip, 182 were induced by aphid infestation and 29 were decreased in their expression in a resistant barley line. Among the induced genes, we identified those that were already known from previous studies to be induced by aphids (chitinases, β-1,3-glucanases, and other PR proteins), in addition to a number of genes that are up to now not implicated in aphid responses. These results will be compared with those from a susceptible barley line.

Keywords: Barley, Micoarray, *Rhopalosiphum padi*, Methyl transferase

P2.28
MicroRNAs may be transported in phloem

P.M.C. Smith, M. Jordan, A.J. Mann and C.A. Atkins, (School of Plant Biology, The University of Western Australia, Crawley, WA, Australia; pmsmith@cyllene.uwa.edu.au)

MicroRNAs (miRNAs) are a recently discovered class of small non-coding RNA that play a significant regulatory role in both plants and animals by targeting mRNAs for cleavage or translational repression. In plants, many of the targets of miRNAs are mRNAs encoding transcription factors that play a role in developmental processes, but it is becoming obvious that they regulate many processes and may also have a role in regulating plant responses to stress. Given the similarity of miRNAs to siRNAs, which generate systemic RNA silencing in plants, it is conceivable that miRNAs act as a signal molecule, translocated in

the phloem to distant organs, allowing the plant to respond to environmental stimuli sensed in a different part of the plant. We are investigating the transport of miRNAs in *Lupinus albus*, a plant from which phloem exudate can easily be isolated. The presence of miRNAs in phloem was confirmed through both cloning and hybridisation approaches. Many of the miRNAs were abundant in phloem exudates and were also found in many different plant tissues. A number of miRNAs had different abundances in phloem isolated from different parts of the plant, suggesting that if they are transported, their transport may not be directly related to the source–sink relationships of the plant.

P2.29
Of lice and plants: metabolite flows in the trophic pathway of phloem-feeding insects as analysed through genomic and EST data from the aphid symbiosis

Y. Rahbé[a], F. Calevro[a], H. Charles[a], G. Febvay[a] and D. Tagu[b], ([a]UMR 203 INRA/INSA de Lyon BF2I, Biologie Fonctionnelle, Insectes et Interactions, INSA, Bât. Louis Pasteur, 69621 Villeurbanne Cedex, France; [b]UMR 1099 INRA/Agrocampus Bio3P, Biologie des Organismes et des Populations Appliquée à la Protection des Plantes, Domaine de la Motte au Vicomte, 35653 Le Rheu Cedex, France; yvan.rahbe@jouy.inra.fr)

Phloem-feeding insects exploit a trophic niche cumulating specific attributes. Unlike blood-feeding insects with which they share vessel-enclosed diets (and thus adaptive specialised mouthparts), they feed on tissues deficient in many common animal nutrients, such as lipids, proteins, some vitamins, and essential amino acids. Due to their specific location in the carbon and nitrogen cycles, plant vessels display compositional biases to which their insect parasites adapted through hundreds of MY of coevolution, including recurrent symbiotic events within the hemipteran lineage. Within the trophic pipeline, leading metabolites from autotrophic (plant and microbial) source cells to auxotrophic (animal) sinks, biosynthetic enzymes, and transporter systems constitute key components of these metabolic flows. With the advent of genomic data at both the microbial and the insect sides of this chain (*Buchnera* and *Acyrthosiphon pisum*), it becomes possible to sketch synthetic views of metabolite partitioning at the sink end of the phloem–insect system. From the genomic and early transcriptome analysis of *Buchnera*, and from EST analysis of the aphid tissues involved in this system (midgut and bacteriocytes), we will point out key biosynthesis and transport questions, which echo those raised by plant physiologists at the source side of this system. The contribution of coupled host–symbiont transcriptomics to these questions will be discussed.

Keywords: Pea aphid, Symbiosis, Transport, Expressed sequence tags, Amino acids

P2.30
Examination of plant signalling pathways and expression profiling of defence genes in *Medicago truncatula* cultivars with resistance to multiple aphid species

L.-L. Gao[a,b], J.P. Anderson[a], J. Klingler[b], R. Horbury[b], R.M. Nair[c], O.R. Edwards[b] and K.B. Singh[a], ([a]CSIRO Plant Industry and [b]CSIRO Entomology, Private Bag 5, Wembley, WA 6913, Australia; [c]South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001, Australia; lingling.gao@csiro.au)

Aphids are serious pests of legumes and other crops worldwide. In contrast to plant–pathogen interactions, very little is known about molecular mechanisms underlying plant resistance to aphids. Australian breeders have introgressed *Acyrthosiphon kondoi* (bluegreen aphid) resistance into three popular cultivars and generated three pairs of resistant and susceptible near-isogenic lines in the model legume *Medicago 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 a single dominant gene conditions resistance to bluegreen aphid. Jester also exhibits, to different degrees, resistance to pea aphid, spotted alfalfa aphid, and spotted clover aphid. Analysis of the transcriptional changes in defence-related genes representing various signaling pathways suggests that both salicylic acid and jasmonic acid pathways are involved in the resistance of Jester to bluegreen aphid. We are currently taking high-throughput approaches to analyse gene expression patterns during various compatible and incompatible aphid interactions in order to further understand this important plant–insect interaction.

Keywords: Medicago truncatular, Aphid resistance, Signalling pathway, Plant-aphid interaction

P2.31
Systemic response to aphid infestation by *Myzus persicae* in the phloem of *Apium graveolens*

F. Divol, F. Vilaine, S. Thibivilliers, J. Amselem, J.C. Palauqui, C. Kusiak and S. Dinant, (Laboratory of Cell Biology, INRA, Versailles 78026, France; dinant@versailles.inra.fr)

We investigated molecular responses to aphid feeding on celery (*Apium graveolens* cv Dulce) plants infested with the aphid *Myzus persicae*, as a means of identifying changes in phloem function. We used celery as our model species as it is easy to separate the phloem from the surrounding tissues in the petioles of mature leaves of this species. We generated a total of 1187 expressed sequence tags (ESTs), corresponding to 891 non-redundant genes. We analysed these ESTs in silico after cDNA macroarray hybridisation. Aphid feeding led to significant increase in RNA accumulation for 126 different genes. Different patterns of deregulation were observed, including transitory or stable induction 3 or 7 days after infestation. The genes affected belonged to various functional categories and were induced systemically in the phloem after infestation. In particular, genes involved in cell wall modification, water transport, vitamin biosynthesis, photosynthesis, carbon assimilation, and nitrogen and carbon mobilisation were up-regulated in the phloem. Further analysis of the response in the phloem or xylem suggested that a component of the response was developed more specifically in the phloem. However, this component was different from the stress responses in the phloem driven by pathogen infection. Our results indicate that the phloem is actively involved in multiple adjustments recruiting metabolic pathways and in structural changes far from aphid feeding sites. However, they also suggest

that the phloem displays specific mechanisms that may not be induced in other tissues.
Keywords: EST, Compatible interaction, Plant defence, Transcriptome

P2.32
Proteins expressed in wheat (*Triticum aestivum*) in response to Russian wheat aphid (*Diuraphis noxia*) infestation

R.A. van Zyl, P. Mathabe, C. Hlongwane, L. Bahlmann, and A.-M. Botha, (Department of Genetics, Forestry and Biotechnology Institute (FABI), University of Pretoria; Bio/Chemtek, CSIR, Pretoria, South Africa; ambothao@postino.up.ac.za)

Two near-isogenic wheat lines (NILs), 'Tugela' (susceptible) and 'TugelaDN' (resistant, *Dn1*), were infested with cereal pest, Russian wheat aphid (*Diuraphis noxia* Mordvilko) Proteins were extracted from the apoplast and simplast of the NILs, prior and post-infestation, and visualized with SDS-PAGE and two-dimensional gel electrophoresis (pH range 4–10). Comparing the apoplastic profiles of the infested and uninfested NILs yielded 10 differences in response to RWA infestation: five proteins (≈36, 26, 20, and two <14.2 kDa) were newly expressed, two were absent (≈29 and 24 kDa), two were induced (22 and ≈45 kDa), and one was down-regulated (≈40 kDa). One of the overexpressed bands (22 kDa) was further resolved into three proteins (pI 4.8, 5.2, and 5.6). In addition to this, two proteins were absent (≈17 and <14.4 kDa) and one was newly expressed (≈24 kDa) in the simplast.
Keywords: Wheat, Russian Wheat Aphid, 2D Gel electrophoresis

P2.33
Transcript profiles induced by aphid feeding in tomato foliage

S. Hebert and F.L. Goggin, (University of Arkansas, Fayetteville, AR, USA)

The majority of studies on plant resistance to insects have focused on chewing insects, such as caterpillars, which cause extensive mechanical damage and induce many wound-responsive and defense-related genes. Piercing–sucking insects that feed from vascular tissue, such as aphids, cause much less wounding than chewing insects, and may induce a different suite of plant responses. In this study, microarray analysis was performed to identify genes that are differentially expressed in tomato foliage when plants were challenged with a piercing–sucking insect (the potato aphid, *Macrosiphum euphorbiae*) or a chewing insect (the beet armyworm, *Spodoptera exigua*). Tissue samples were collected at 6, 12, and 24 h, and control plants (no insect challenge) were compared to samples challenged with either aphids or armyworms separately using a cDNA microarray that is estimated to represent approximately 25% of the tomato genome. Our results indicate that numerous genes involved in signal transduction, defense, stress responses, cell maintenance, and development are differentially regulated in response to insect herbivory. Fewer than 3% of these differentially regulated genes are responsive to both aphids and caterpillars, which supports the hypothesis that plant responses to piercing–sucking insects are unique from responses to chewing insects. Furthermore, analysis of tomato lines with deficiencies in defensive signaling suggests that there may be trade-offs between plant defenses against these different insect-feeding guilds.

P2.34
Examination of plant signalling pathways and expression profiling of defence genes in *Medicago truncatula* cultivars with resistance to multiple aphid species

L.-L. Gao[a,b], J.P. Anderson[a], J. Klingler[b], R. Horbury[b], R.M. Nair[c], O.R. Edwards[b] and K.B. Singh[a], ([a]CSIRO Plant Industry and [b]CSIRO Entomology, Private Bag 5, Wembley, WA 6913, Australia; [c]South Australian Research and Development Institute, GPO Box 397, Adelaide, SA 5001, Australia; lingling.gao@csiro.au)

Duplicate

P2.35
Exploring plant–aphid interactions using transcriptomics and a phloem cDNA library

C.E. Couldridge[a], J.S. Bale[a], C. Doering-Saad[a], H.J. Newbury[a], D. O'Reilly[b] and J. Pritchard[a], ([a]School of Biosciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK; [b]Syngenta, Jealotts Hill Research Centre, Bracknell, Berkshire RG42 6EY, UK)

Interactions between herbivores and their host plants are central to many ecological and agricultural situations. An important herbivore cohort includes sap-sucking insects such as aphids, which are pests of many commercial crops. Their pest status is predicted to increase as global warming continues to alter weather patterns and pest ranges. Changes in gene expression often occur in the host during feeding. These changes maybe representative of a general damage response and can occur specifically targeted to the phloem feeding site. Alternatively, changes in gene expression maybe induced by the insect to manipulate the plant to produce a more favourable diet or overcome a plant defence. Thus, understanding changes in plant gene expression occurring in response to aphid feeding will give valuable insights into the fundamental biology of the plant–insect interaction, as well as informing biotechnology strategies for novel approaches to aphid control.
This study uses two related approaches to study plant gene expression. Firstly, transcriptomics have been used to examine changes in gene expression in *Arabidopsis thaliana* leaves in response to feeding by the peach–potato aphid, *Myzus persicae* after 2 and 36 h of infestation. Secondly, a phloem cDNA library from *Ricinius communis* has been analysed to develop a better understanding of the genes involved in phloem function, the organ in which aphids feed. Both have the goal of identifying potential gene candidates for manipulation in future projects to study the plant–aphid interaction in more detail.
Keywords: Plant-aphid interaction, Transcriptomics, Phloem cDNA library, *Arabidopsis thaliana*, *Myzus persicae*

P2.36
Studies on feeding site preferences of aphids on tansy: a combination of morphological, physiological, and behavioural investigations

S.N. Bauer and E. Komor, (University of Bayreuth, Department of Plant Physiology, D-95440 Bayreuth, Germany; susanne.bauer@uni-bayreuth.de)

Tansy (*Tanacetum vulgare* L. (Asteraceae)) is the host plant for a guild of aphid species that utilize specific feeding niches (e.g., fresh shoots, stems, senescing leaves, or the leaf margins). Several anatomical, morphological, and physiological factors, which likely contribute to the aphid species' spatial distribution pattern, were investigated in this study.

The distance of the phloem from the surface in stems and leaves of flowering tansy plants was measured from transverse sections and compared with the stylet lengths of aphid species colonizing these plant parts. The short stylet length of the smallest monophagous aphid species (*Coloradoa tanacetina*) correlated well with the low phloem depth at its feeding sites (flower stalks and minor leaf veins), while no correlations were found for larger aphid species.

Since the nutritional quality of the phloem sap, in particular its nitrogen content, is hypothesised to affect aphid performance and their acceptance of a feeding site, amino acid analyses of plant tissue extracts and exudates from excised leaves in EDTA solution were carried out. Interestingly, the amino acid composition showed a higher variation between plant individuals than between different parts of a plant. However, further investigations on phloem sap composition are necessary to draw conclusions on correlations between plant amino acid profiles and aphids' feeding site choice.

The feeding behaviour of several aphid species on different plant parts was recorded by the EPG (electrical penetration graph) technique. Preliminary results suggest differences in the feeding behaviour on leaves of different age.

Keywords: Stylet length, Amino acid composition, Electrical penetration graph

P2.37
Real-time PCR for detection of apple proliferation phytoplasma in host plants and vectors

S. Baric, C. Kerschbamer and J.G. Dalla Via, (Research Centre for Agriculture and Forestry Laimburg, 39040 Auer/Ora, Italy; sanja.baric@provinz.bz.it)

Apple proliferation is an incurable disease of apple trees, which can induce considerable economic losses by reducing quality, size, and overall yield of fruit. The causal agent, apple proliferation (AP) phytoplasma, is restricted to the phloem tissue of the host plant and was shown to be transmitted by sap-sucking insects of the genus *Cacopsylla*. So far, there are many gaps in the understanding of the disease caused by the fact that phytoplasmas are obligatory endocellular organisms that cannot be cultured in vitro on artificial media. Thus, the availability of diagnostic techniques allowing reliable and sensitive detection of the pathogen in host plants and insect vectors is of enormous importance. In the present study, a recently developed real-time PCR assay for the detection of AP phytoplasma (1) was compared to four conventional PCR diagnostic procedures by parallel testing of DNA extracts obtained from field samples of apple trees and the psyllid *Cacopsylla melanoneura*. Our data show that apart from its high specificity and rapidity, real-time PCR is characterized by an outstanding sensitivity. This can be relevant when testing asymptomatic latently infected apple trees, but seems to be crucial when investigating insect vectors having a low titer of AP phytoplasma. In addition, the future development of a quantitative PCR assay could provide novel insights into the transmission mechanisms of the pathogen and its dynamics in host plants, and so contribute to devise new measures for effective disease control.

Keywords: Diagnosis, Plant disease, *Malus domestica*, Psyllid

(1) S. Baric, J. Dalla Via. 2004. *J. Microbiol. Methods* 57: 135–145.

P2.38
Abstract withdrawn

P2.39
Influence of the octadecacanoid signaling pathway on plant–aphid interactions

Lingling Jia, Stephanie Hebert, and Fiona L. Goggin, (University of Arkansas, Fayetteville, AR, USA; fgoggin@uark.edu)

Jasmonic acid and its derivatives are induced in response to wounding, and act as local and systemic signals that trigger expression of proteinase inhibitors, polyphenol oxidase, and other compounds that have a defensive function against many insect herbivores. Jasmonates are known to mediate induced resistance against many chewing insects such as caterpillars and cell content feeders such as mites and thrips. It is not known, however, if this pathway is responsive to or effective against piercing–sucking insects such as aphids, which cause far less mechanical damage than other insect feeding guilds. This study assessed population growth of the potato aphid, *Macrosiphum euphorbiae*, on a tomato mutant that is deficient in jasmonic acid synthesis, and also quantified expression of marker genes associated with jasmonate induction in response to aphid feeding.

Keywords: Jasmonic acid, Induced resistance

P2.40
Using aphid stylectomy to investigate systemicity of applied chemicals

M. Daniels[a], H.J. Newbury[a], J.S. Bale[a], R. Lind[b], J. Pritchard[a], ([a]The University of Birmingham, Edgbaston, Birmingham B15 2TT; [b]Syngenta, Jealott's Hill International Research Centre, Bracknell, RG42 6EY; mxd015@bham.ac.uk)

Aphids are major pests of many agricultural crops, causing damage by transmission of viruses and direct feeding damage. Over the last sixty years, insecticides have played a key role in controlling these pests. However, owing to safety concerns and resistance emerging in many insects, there is a continual need to search for novel approaches. An important aspect in developing new insecticides is to understand the translocation of a compound through the plant to the insect. As aphids are sap sucking pests, feeding primarily from the phloem, the systemicity of a compound is an important property to be considered when developing new insecticides. This allows insecticides to be delivered to the point of aphid feeding.

Reported here is a method for studying systemicity of compounds using aphid stylectomy. Stylectomy is an effective method of collecting phloem sap whilst avoiding contamination from surrounding cells. The stylet of a feeding aphid is cut using a high frequency microcautery unit and the resultant exuding sap collected. Using the agriculturally important monocot, wheat (*Triticum aestivum* cv. Paragon), the systemicity of compounds was studied using the bird cherry–oat aphid (*Rhopalosiphum padi*) to perform stylectomy. Exogenously applied ^{14}C labelled compounds were detected in phloem sap using liquid scintillation counting, indicating that the compounds were phloem mobile. Reported here are preliminary findings of the relative phloem mobility of various ^{14}C labelled compounds.

Keywords: Aphid, Phloem, Stylectomy, Systemicity, Insecticide

P2.42
Perception of aphid infested tomato plant volatiles by the predator *Episyrphus balteatus*

François Verheggen, Ludovic Arnaud, Quentin Capella, Frédéric Francis and Eric Haubruge, (Department of Functional and Evolutionary Entomology, Gembloux Agricultural University, Passage des Déportés 2, 5030 Gembloux, Belgium; verheggen.f@fsagx.ac.be)

In a tritrophic interaction including tomato plant (*Lycopersicon esculentum* Miller), the herbivore *Myzus persicae* (Sulzer), and the predator *Episyrphus balteatus* (De Geer), the perception of the tomato plants produced volatile organic compounds (VOC) by *E. balteatus* is investigated. In a first step, an odour sampling device has been set up, aiming at the headspace collection of the tomato plant VOC and their adsorption on Tenax adsorbent cartridges (Supelco®). The following desorption is held using a thermodesorption injector (Gerstel®) coupled with GC-MS. Intact and aphid infested plants are studied for their VOC emissions, as well as the comparison of the VOC emission of different tomato cultivars. These VOC consist mainly in mono- and sesquiterpenes (such as α-pinene, β-pinene, α-humulene, etc.) as well as in C_6 volatiles like hexenal in case of infestation by herbivores. Once the tomato plants' VOC are identified and quantified, they are tested for their perception by *E. balteatus* using electroantennography (EAG). Accordingly, an EAG device has been installed and configured for the study of VOC using Diptera antennas. The monoterpenes limonene and linalool showed high EAG activity, whereas other terpenes like cymene seem to be inactive.

P2.43
Proteomic study of plant–aphid interactions: between defence and adaptations

Frédéric Francis[a], Pascal Gerkens[b], Edwin Depauw[b] and Eric Haubruge[a], ([a]Functional and Evolutionary Entomology Unit, Gembloux Agricultural University; [b]Mass Spectrometry Laboratory; University of Liège)

Chemical ecology is the study of how particular chemicals are involved in interactions of organisms with each other and with their surroundings. In order to reduce insect attack, plants have evolved a variety of defence mechanisms, both constitutive and inducible, while insects have evolved strategies to overcome these plant defences (such as detoxification enzymes). A major determinant of the influence of evolutionary arms races is the strategy of the insect: generalist insect herbivores, such as *Myzus persicae* aphid, need more complex adaptive mechanisms since they need to respond to a large array of different plant-defensive chemicals. Here we studied the chemical ecology of *M. persicae* associated with plant species from Solanaceae family. To identify the involved adaptation systems to cope with the plant secondary substances and to assess the differential expression of these systems, a nonrestrictive proteomic approach was developed to identify all the potential adaptation systems toward the secondary metabolites from host plants. The complex protein mixtures was separated by two-dimensional electrophoresis methods, and the related spots of proteins varying at least with a twofold ratio were selected and identified by mass spectrometry (MALDI TOF) coupled with data bank investigations. These techniques are very reliable to describe the proteome from organisms such as insects, in response to particular host plant defence mechanisms.