Comparisons of Salivary Proteins From Five Aphid (Hemiptera: Aphididae) Species

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ABSTRACT Aphid (Hemiptera: Aphididae) saliva, when injected into host plants during feeding, causes physiological changes in hosts that facilitate aphid feeding and cause injury to plants. Comparing salivary constituents among aphid species could help identify which salivary products are universally important for general aphid feeding processes, which products are involved with specific host associations, or which products elicit visible injury to hosts. We compared the salivary proteins from five aphid species, namely, *Diuraphis noxia* (Kurdjumov), *D. tritici* (Gillette), *D. mexicana* (Baker), *Schizaphis graminum* (Rondani), and *Acyrthosiphon pisum* (Harris). A 132-kDa protein band was detected from the saliva of all five species using sodium dodecyl sulfate polyacrylamide gel electrophoresis. Alkaline phosphatase activity was detected from the saliva of all five species and may have a universal role in the feeding process of aphids. The *Diuraphis* species cause similar visible injury to grass hosts, and nine electrophoretic bands were unique to the saliva of these three species. *S. graminum* shares mutual hosts with the *Diuraphis* species, but visible injury to hosts caused by *S. graminum* feeding differs from that of *Diuraphis* feeding. Only two mutual electrophoretic bands were visualized in the saliva of *Diuraphis* and *S. graminum*. Ten unique products were detected from the saliva of *A. pisum*, which feeds on dicotyledonous hosts. Our comparisons of aphid salivary proteins revealed similarities among species which cause similar injury on mutual hosts, fewer similarities among species that cause different injury on mutual hosts, and little similarity among species which feed on unrelated hosts.

KEY WORDS Russian wheat aphid, western wheat aphid, grass aphid, greenbug, pea aphid

Aphids (Hemiptera: Aphididae) are important pests of crops worldwide. Aphids ingest phloem sap through stylet mouthparts that penetrate intercellular plant tissues and tap the phloem sieve-tube. While feeding, aphids inject two types of saliva into host-plant tissues: the salivary sheath (gel) and soluble (watery) saliva (Miles 1959). Sheath saliva hardens upon secretion to become an insoluble lining of the stylet path and is thought to suppress plant defenses (Miles 1999). Soluble saliva is involved in establishing and maintaining feeding sites, suppressing plant defenses, and/or inducing changes in plant physiology that facilitate aphid feeding and improve the nutritional quality of hosts (Miles 1999, Will et al. 2007, Mutti et al. 2008). Furthermore, aphid saliva is likely important to host-plant specificity among aphid species and elicitation of injury or defense responses from host plants (Miles 1999 and references therein).

Because of its role in aphid-plant interactions, interest in the composition of aphid saliva has recently increased (Miles 1999, Cherqui and Tjallingii 2000, Will et al. 2007, Carolan et al. 2009, Cooper et al. 2010). Salivary proteins have been directly investigated from the aphids *Megoura viciae* Buckton, *Acyrthosiphon pisum* (Harris), *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas), *Aphis fabae* Scopoli, *Nasonovia ribisnigri* (Mosely), *Sitobion avenae* (F.), *Schizaphis graminum* (Rondani), and *Diuraphis noxia* (Kurdjumov) (Miles and Harrewijn 1991, Baumann and Baumann 1995, Urbanska et al. 1998, Cherqui and Tjallingii 2000, Will et al. 2007, Harmel et al. 2008, Carolan et al. 2009, De Vos and Jander 2009, Cooper et al. 2010). Other studies have investigated proteins from the salivary gland extracts of *A. pisum*, *D. noxia*, and *Rhopalosiphum padi* L. (Ni et al. 2000, Mutti et al. 2006, Mutti et al. 2008). Although the saliva and salivary gland extracts of a diversity of aphid species have been investigated, very few studies have directly compared the salivary profiles of different aphid species (but see Miles and Harrewijn 1991, Cherqui and Tjallingii 2000). Direct comparisons of salivary constituents among aphid species could help identify salivary proteins that may be universal among aphids and that

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have general roles in the feeding process, or that are unique to specific host associations or which cause specific injury to hosts.

The goals of our study were to investigate variations among the salivary proteins of three *Diuraphis* species, *S. graminum*, and *A. pisum*. *D. noxia* (Russian wheat aphid) was introduced into the United States in 1986, and is a pest of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Morrison and Peairs 1998). Cultivated grasses are the preferred hosts of *D. noxia*, but this species also uses certain wild grasses as hosts (Springer et al. 1992). *D. tritici* (Gillette) (western wheat aphid) and *D. mexicana* (Baker) are both native to North America. *D. tritici* is an occasional pest of wheat in the United States. *D. mexicana* uses wild grasses as hosts, and is an occasional pest of the forage crop 'Garnett' mountain brome, *Bromus marginatus* Ness ex Steud. (Hammon and Peairs 1998). Symptoms on grass hosts caused by feeding are similar among these three *Diuraphis* species. *S. graminum* is another important pest of wheat and barley, but injury caused by *S. graminum* feeding is different from that caused by *Diuraphis* feeding. *A. pisum* uses dicotyledonous plants as hosts, and was included in our study because it is used as a model organism for aphid-host interactions (Tagu et al. 2008) and its genome has recently been sequenced (International Aphid Genomics Consortium 2010). The salivary protein profiles of these aphid species were compared to assess their similarity among aphids with shared host preferences or among aphids that cause similar injury to mutual hosts. Additionally, we compared the salivary proteins of aphids with distinctly different host preferences to identify whether specific proteins are shared among unrelated aphids that use different hosts. We postulated the salivary protein profiles would be more similar among aphid species that cause visibly similar injury to mutual hosts compared with aphid species that cause visibly different injury to mutual hosts or with aphid species which differ in host preference.

Methods and Materials

Aphid Rearing and Collection. All aphid species used in our study were mass reared under controlled conditions (16:8 L:D h, 23–25°C). *D. noxia* (USA-biotype-1), *D. tritici*, *D. mexicana*, and *S. graminum* (biotype E) were reared on Yuma and Yumar wheat cultivars, TAM110 wheat line, 'Garnett' Mountain Brome, and '812′ barley line, respectively. Grass feeding aphids and their hosts were enclosed in clear plastic cylinder cages with fine-mesh cloth windows. *A. pisum* were reared on faba bean, *Vicia faba* L., within an insectary at Oklahoma State University. Large numbers of aphids were collected from heavily infested plants by gentle shaking, and aphid numbers were estimated based on weight.

Saliva Collection and Concentration. Saliva collection plates were prepared with 15% sucrose diet as described by Cooper et al. (2010). Collection plates were prepared under sterile conditions by stretching Parafilm M (Pechiney Plastic Packaging, Chicago, IL) to 3× its original size over the bottom of sterile 100 × 15-mm petri dishes (actual diameter is 90 mm top, 87 mm bottom) with beveled stacking ridges on their bases (Fisher, Pittsburgh, PA, cat. no. 08–757-13). The shallow dishes created by the stacking ridges were filled with 4 ml of 15% sucrose, which was evenly spread under the Parafilm. Preparation of the collection plates and diet were performed under a sterile laminar flow hood, and materials were sterilized by UV-radiation. Aphids (≈450 per plate) were placed on the Parafilm surface and covered with the petri dish lid. Two 0.75-mm thick plastic rings (90 mm o.d., 86 mm i.d.) cut to fit the i.d. of the petri dish lids were used to provide minimal vertical space for the aphids. Collection plates prepared without aphids were used as a control to monitor contamination. The saliva collection plates were placed between horizontal sheets of yellow paper within growth chambers (16:8 L:D h, 20°C). After 24 h, diet was pooled from 25 feeding plates (≈11,000–12,000 aphids), and salivary proteins were concentrated to 80 μl using 3-kDa cutoff centrifuge concentrators (VivaSpin 20 and VivaSpin 2, Satorius Group, Goettingen, Germany). Salivary protein concentrations were estimated using a Bradford protein assay kit (Pierce Scientific, Rockford, IL) with bovine serum albumin as a standard. Saliva was collected and analyzed twice from each aphid species.

Protein Electrophoresis. One dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 10% Tris-HCl precast gels (Bio-Rad Laboratories, Hercules, CA) in a MiniProtean Electrophoresis Unit (Bio-Rad Laboratories) at 30 mA for 1 h. Sample buffer consisting of 0.25 M Tris-HCl pH 6.8, 20% glycerol, 5% SDS, and 0.4 M dithiothreitol was added to each sample (0.7 μg of total protein), and sample solutions were held at 95°C for 5 min before SDS-PAGE. Two-dimensional SDS-PAGE of salivary proteins was performed within a Bio-Rad Protean IEF Cell (Bio-Rad Laboratories) using 7-cm 3–10 linear GE Immobiline Drystrips (GE Lifesciences, Pittsburgh, PA). Strips were rehydrated for 18 h with 120 μl urea/thiourea rehydration buffer with 2% Triton X-100, 80 mM dithiothreitol, and 1.5 μg of salivary protein. The isoelectric focusing conditions were 200 V for 200 V-h, 500 V for 500 V-h, 1000 V for 100 V-h, and 8,000 V for 60,000 V-h. The second dimension was carried out on 10% precast gels (Bio-Rad Laboratories) at 5 mA for 15 min followed by 15 mA for 2 h. Gels were stained with silver stain to visualize protein bands. Reagents for two dimensional SDS-PAGE and gel staining were obtained from GE Lifesciences (Pittsburgh, PA).

Alkaline Phosphatase Detection. The AttoPhos AP Fluorescence Substrate System (Promega Corporation, Madison, WI) was used to assess alkaline phosphatase activity from each aphid species (0.7 μg salivary protein). Samples were prepared in nonfluorescent assay plates by adding samples to 160 μl of phosphatase buffer and incubating for 30 min at room temperature. Fluorescence was measured using a Typhoon Trio Image Scanner (GE Lifesciences, Pittsburgh, PA) in fluorescence acquisition mode with a

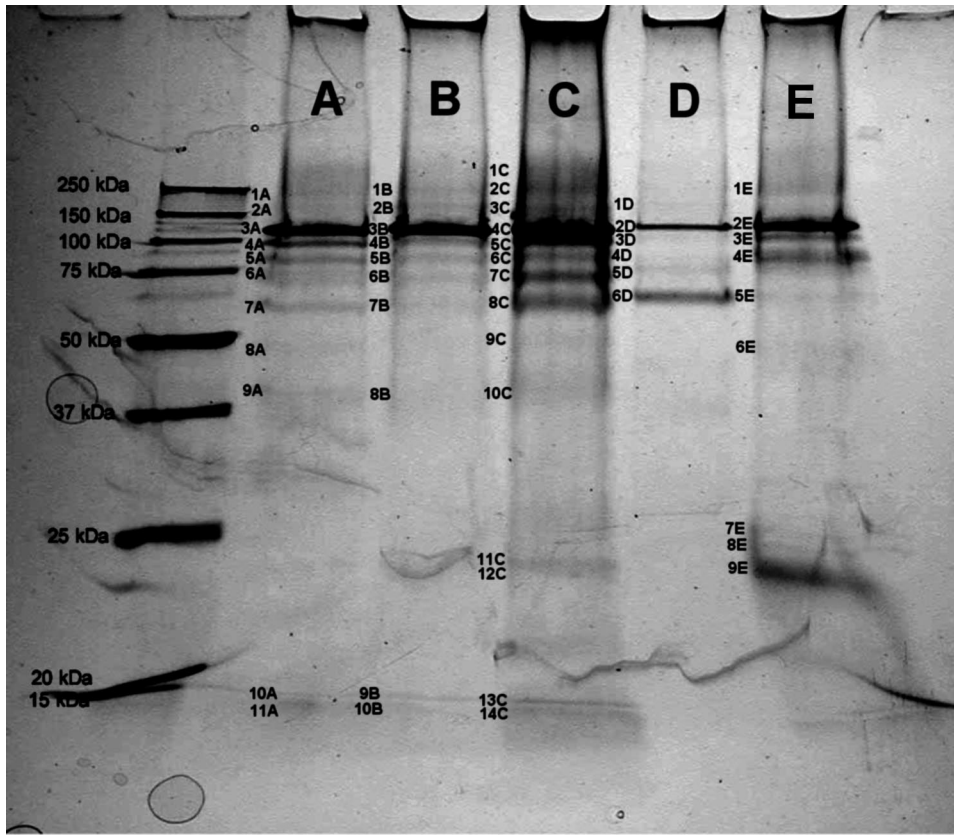

Fig. 1. Electrophoretic separation of salivary proteins (0.7 μg total protein per lane) from (A) *D. noxia*, (B) *D. tritici*, (C) *D. mexicana*, (D) *S. graminum*, and (E) *A. pisum*. The estimated size of each band is presented in Table 1.

488-nm excitation laser set at high intensity, and a 580-nm emission filter.

Results and Discussion

Our saliva collection methods using a 15% sucrose solution (Cooper et al. 2010) collected adequate amounts of saliva from each aphid species to compare the electrophoretic profiles of abundant salivary proteins (Figs. 1 and 2). We collected 0.28 ng salivary protein/aphid (bovine serum albumin equivalents) from *D. noxia*, which was consistent with our previous study (Cooper et al. 2010). We collected 0.22 ng salivary protein/aphid from *D. mexicana*, 0.51 ng salivary protein/aphid from *D. tritici*, 0.63 ng salivary protein/aphid from *S. graminum*, and 0.31 ng salivary protein/aphid from *A. pisum.* Protein losses during collection and concentration were assumed, thus values do not reflect absolute amounts secreted by individual aphids. Protein was not detected from our control samples. This study demonstrates that our method developed for collecting *D. noxia* saliva (Cooper et al. 2010) is also suitable for other aphid species, thus providing a simple and inexpensive method of collecting aphid saliva. Furthermore, use of a simple feeding solution of 15% sucrose provided repeatable results and the proteins were easily concentrated. However, the use of different dietary substrates may cause certain aphids to secrete different salivary protein profiles (Miles 1999, Cooper et al. 2010).

As many as 25 different protein bands were collectively visualized from the five aphid species using SDS-PAGE (Table 1; Fig. 1). Although electrophoresis will not detect all proteins present in salivary samples, it allows comparison of the abundant protein products in the saliva from different species. Electrophoresis revealed similarities in protein banding patterns among each aphid species. Gels from all five species indicated a single mutual protein band with an estimated molecular weight of 132 kDa, (bands 3A, 3B, 4C, 2D, and 2E; Table 1, Fig. 1). A previous study of *D. noxia* saliva suggested this band was comprised of alkaline phosphatase, a RNA helicase-like protein, a dehydrogenase-like protein, and at least two other unidentified products (Cooper et al. 2010). We detected alkaline phosphatase activity in the saliva of all five aphid species (Fig. 3). Although alkaline phosphatases were previously detected in the saliva of *D. noxia* (Cooper et al. 2010) and sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Funk 2001), phosphatase activity was not detected in the saliva of yellow rose aphid, *A. porosum* (Sanderson), or cotton aphid, *Aphis gossypii* Glover (Funk 2001). However, the focus of the study by Funk

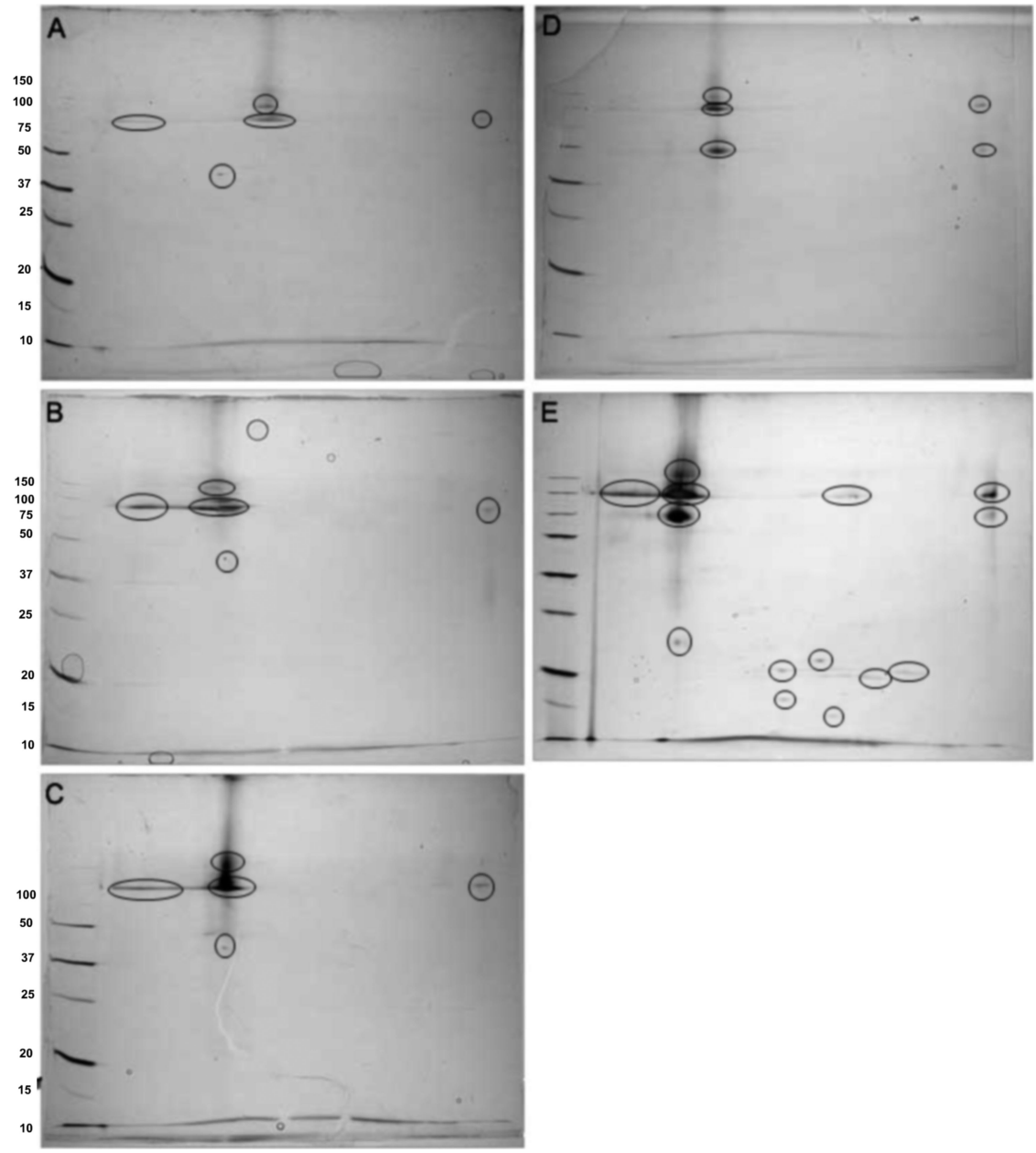

Fig. 2. Salivary proteins separated on 3–11-NL Biorad isolectric focusing strips (first dimension) and 10% SDS-PAGE (second dimension). The first dimension is arranged with pH three on the left side of each gel. (A) *D. noxia*, (B) *D. tritici*, (C) *D. mexicana*, (D) *S. graminum*, and (E) *A. pisum.*

(2001) was whitefly saliva, and consequently, detailed aphid data may be lacking. Alkaline phosphatases are present in whitefly glandular tissues which produce hardened sclerotized structures. Those tissues include the salivary glands, colleterial glands, and ovarioles, which produce salivary sheaths, the substance used to attach eggs to substrates, and the egg chorion, respectively (Funk 2001). Alkaline phosphatases may be important to the sclerotization of the salivary sheath (Funk 2001, Cooper et al. 2010), but we cannot rule out the potential for roles in carbohydrase activities or detoxification of plant defenses. Results from our study suggest that phosphatases are universally important in hemipteran feeding processes, but further studies are required to elucidate the precise roles of alkaline phosphatases in aphid feeding.

The saliva of *D. noxia*, *D. tritici*, and *D. mexicana* shared at least ten products of similar size (Table 1; Fig. 1). Two dimensional SDS-PAGE revealed variations in the 132-kDa products collected from the saliva

Table 1. Estimated molecular weights of protein bands visualized on SDS-PAGE representing the concentrated salivary constituents of five aphid species collected on 15% sucrose diet

D. noxia	*D. tritici*	*D. mexicana*	*S. graminum*	*A. pisum*
—	—	1C: 325 kDa	—	—
—	—	—	—	1E: 244 kDa
1A: 227 kDa	1B: 227 kDa	2C: 227 kDa	—	—
2A: 176 kDa	2B: 176 kDa	3C: 176 kDa	1D: 176 kDa	—
3A: 132 kDa	3B: 132 kDa	4C: 132 kDa	2D: 132 kDa	2E: 132 kDa
—	—	—	3D: 113 kDa	3E: 113 kDa
4A: 108 kDa	4B: 108 kDa	5C: 108 kDa	—	—
—	—	—	4D: 94 kDa	—
5A: 90 kDa	5B: 90 kDa	6C: 90 kDa	—	4E: 90 kDa
—	—	—	5D: 83 kDa	—
6A: 77 kDa	6B: 77 kDa	7C: 77 kDa	—	—
—	—	—	6D: 64 kDa	—
—	—	—	—	5E: 62 kDa
7A: 60 kDa	7B: 60 kDa	8C: 60 kDa	—	—
—	—	9C: 47 kDa	—	—
8A: 45 kDa	—	—	—	6E: 45 kDa
9A: 38 kDa	8B: 38 kDa	10C: 38 kDa	—	—
—	—	—	—	7E: 26 kDa
—	—	—	—	8E: 25 kDa
—	—	—	—	9E: 23 kDa
—	—	11C: 18 kDa	—	—
—	—	12C: 15 kDa	—	—
10A: 10 kDa	9B: 10 kDa	13C: 10 kDa	—	—
11A: 8 kDa	10B: 8 kDa	14C: 8 kDa	—	—

Band labels correspond with the labels on Fig. 1.

of these three *Diuraphis* species (Figs. 2A–C). Specifically, two products from within the 132-kDa band from *D. noxia* saliva focused closer to an intermediate pI compared with the equivalent products from the saliva of the other two *Diuraphis* species (Figs. 2A–C). Furthermore, a 45-kDa product (band 8A; Table 1, Fig. 1) was visualized from *D. noxia* saliva that was not detected in the saliva of the other *Diuraphis* species, but a product of similar size was visualized from *A. pisum* saliva (band 6E; Table 1, Fig. 1). We did not detect unique products from the saliva of *D. tritici*, and the salivary protein profile of *D. tritici* was most similar to that of *D. noxia.* Both species use wheat and wild grasses as hosts, and both species cause leaf rolling and chlorotic streaking on hosts (Armstrong et al. 1991, Kindler and Hammon 1996). These similarities in host-use by *D. noxia* and *D. tritici* and the similar appearance of the injury they produce might be explained by the observed similarities in their salivary protein profiles. The salivary protein profile of *D. mexicana* was similar to that of *D. noxia* and *D. tritici*, but several products with estimated molecular weights of 325, 47, 18, and 15 kDa (bands 1C, 9C, 11C, and 12C; Table 1, Fig. 1) were visualized from the saliva of *D. mexicana* that were not detected from the saliva of *D. noxia* or *D. tritici.* Differences in host suitability between *D. mexicana* and the other two *Diuraphis* species might be related to the corresponding differences in salivary protein profiles. Unlike *D. noxia* and *D. tritici*, *D. mexicana* does not use wheat or barley as a host, and its host range is mostly restricted to wild bromes (*Bromus* spp.) (Miller and Stoetzel 2005).

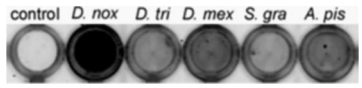

Fig. 3. Alkaline phosphatase activity (indicated by dark coloration within wells) from 0.7 μg of concentrated salivary protein from *D. noxia*, *D. tritici*, *D. mexicana*, *S. graminum*, and *A. pisum*.

Diuraphis spp. and *S. graminum* share mutual hosts, but injury to hosts caused by *S. graminum* feeding is different from that of the three *Diuraphis* species. Specifically, *S. graminum* and *Diuraphis* feeding damage are, respectively, characterized by the development of chlorotic lesions near feeding sites or longitudinal white streaks on infested leaves. Other than the 132-kDa band shared by all five species, these two genera shared only a single product with an estimated molecular weight of 176 kDa (bands 2A, 2B, 3C, and 1D; Table 1, Fig. 1). The 176-kDa product may be important to the use of grass hosts by these four aphid species. Three products with estimated sizes of 94, 83, and 64 kDa (bands 4D, 5D. and 6D; Table 1, Fig. 1) were visualized in the saliva of *S. graminum* but not from the saliva of the other species. Two dimensional SDS-PAGE of *S. graminum* saliva revealed the 132-kDa band (band 2D; Table 1, Fig. 1) shared by all five aphid species was composed of only two products (Fig. 2D) compared with three visualized for each of the *Diuraphis* species (Fig. 2A–C). The observed differences in salivary proteins among *Diuraphis* species and *S. graminum* support the hypothesis that aphid species that cause different injury to mutual hosts have different salivary protein profiles.

Nine bands were detected from *A. pisum* saliva using one dimensional SDS-PAGE and at least 14 products were visualized on two dimensional SDS-PAGE (Table 1; Figs. 1 and 2E). Two dimensional SDS-PAGE revealed the 132-kDa band (band 2E; Fig. 1) shared by

all five aphid species examined in this study was composed of four products in *A. pisum* including a product with an intermediate pI that was not visualized from the saliva of other aphid species (Fig. 2E). Five other protein bands with estimated molecular weights of 244, 62, 26, 25, and 23 kDa (bands 1E, 5E, 7E, 8E, and 9E; Table 1, Fig. 1) were only visualized from *A. pisum* saliva. The profile of salivary proteins of *A. pisum* differed considerably from those of the four grass-feeding aphid species, which may reflect the use of dicotyledonous plants as hosts by *A. pisum* instead of grasses.

Our study is the most comprehensive comparison to date of salivary protein profiles of different aphid species. These comparisons revealed that unrelated aphid species with distinctly different host preferences shared some common salivary protein constituents, including alkaline phosphatases and additional unidentified products within the 132-kDa band. Further characterization of these shared protein products could elucidate general roles of saliva in aphid feeding and host interactions. Overall, our study suggests that aphid species that cause similar injury to mutual hosts have similar salivary protein profiles whereas species that cause different injury to mutual hosts, or species which use different hosts, have different salivary protein profiles. Further investigation of the similarities and differences in salivary constituents among the three *Diuraphis* spp. and between *Diuraphis* spp. and *S. graminum* could lead to a better understanding of host specificity and the role of saliva in causation of visible injury to hosts.

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References Cited

Armstrong, J. S., M. R. Porter, and F. B. Peairs. 1991. Alternate hosts for the Russian Wheat Aphid (Homoptera: Aphididae) in Northeastern Colorado. J. Econ. Entomol. 1691–1694.

Baumann, L., and P. Baumann. 1995. Soluble salivary proteins by *Schizaphis graminum.* Entomol. Exp. Appl. 77: 57–60.

Carolan, J. C., C.I.J. Fitzroy, P. D. Ashton, A. E. Douglas, and T. L. Wilkinson. 2009. The secreted salivary proteome of the pea aphid *Acyrthosiphon pisum* characterized by mass spectrometry. Proteomics 9: 2457–2467.

Cherqui, A., and W. F. Tjallingii. 2000. Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation. J. Insect Physiol. 46: 1177–1186.

Cooper, W. R., J. W. Dillwith, and G. J. Puterka. 2010. Salivary proteins of Russian wheat aphid (*Diuraphis noxia* Kurdjumov, Hemiptera: Aphididae). Environ. Entomol. 39: 223–231.

De Vos, M., and G. Jander. 2009. *Myzus persicae* (green peach aphid) salivary components induce defence responses in *Arabidopsis thaliana.* Plant Cell Environ. 32: 1548–1560.

Funk, C. J. 2001. Alkaline phosphatase activity in whitefly salivary glands and saliva. Arch. Insect Biochem. 46: 165–174.

Hammon, R. W. and F. B. Peairs. 1998. Natural history of *Diuraphis* (Homoptera: Aphididae) species occurring in western Colorado, pp. 412–428. *In* S. S. Quisenberry & F. B. Peairs (eds.), Response Model for an Introduced Pest: The Russian Wheat Aphid. Thomas Say Publications Entomological Society of America, Lanham, MD.

Harmel, N., E. Letocart, A. Cherqui, P. Glordanengo, G. Mazzuccheill, F. Gullionneau, E. De Pauw, E. Haubruge, and F. Fransis. 2008. Identification of aphid salivary proteins: a proteomic investigation of *Myzus persicae.* Insect Mol. Biol. 17: 165–174.

International Aphid Genomics Consortium. 2010. Genome sequence of the pea phid *Acyrthosiphon pisum.* PLoS Biol 8: (e1000313.doi:10.1371/journal.pbio.1000313).

Kindler, S. D., and R. W. Hammon. 1996. Comparison of host suitability of western wheat aphid with Russian wheat aphid. J. Econ. Entomol. 89: 1621–1630.

Miles, P. W. 1959. Secretion of two types of saliva by an aphid. Nature 183: 756–756.

Miles, P. W. 1999. Aphid saliva. Biol. Rev. 74: 41–85.

Miles, P. W., and P. Harrewijn. 1991. Discharge by aphids of soluble secretions into dietary sources. Entomol. Exp. Appl. 59: 123–134.

Miller, G., and M. Stoetzel. 2005. A systematic reappraisal of the genus *Diuraphis* Aizenberg (Hemiptera: Aphididae). Proc. Entomol. Soc. Wash. 107: 700–728.

Morrison, W. P., and F. B. Peairs. 1998. Response model concept and economic impact, pp. 1–11. *In* S. S. Quisenberry & F. B. Peairs (eds.), Response Model for an Introduced Pest: The Russian Wheat Aphid. Thomas Say Publications Entomological Society of America, Lanham, MD.

Mutti, N. S., Y. Park, J. C. Reese, and G. R. Reeck. 2006. RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, *Acyrthosiphon pisum.* J. Insect Sci. 6: (insectscience.org/6.38).

Mutti, N. S., J. Louis, L. K. Pappan, K. Pappan, K. Begum, M.S.C. Ming-Shun, Y. Park, N. Dittmer, J. Marshall, J. C. Reese, and G. R. Reeck. 2008. A protein from the salivary glands of the pea aphid, Acyrthosiphon pisum, is essential in feeding on a host plant. Proc. Nat. Acad. Sci. U.S.A. 105: 9965–9969.

Ni, X., S. S. Quisenberry, S. Pornkulwat, J. L. Figarola, S. R. Skoda, and J. E. Foster. 2000. Hydrolase and oxido-reductase activities in *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae). Ann. Entomol. Soc. Am. 93: 595–601.

Springer, T. L., S. D. Kindler, T. L. Harvey, and P. W. Stahlman. 1992. Susceptibility of brome grass to Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85: 1731–1735.

Tagu, D., J. P. Klingler, A. Moya, and J. C. Simon. 2008. Early progress in aphid genomics and consequences for plant-aphid interactions studies. Mol. Plant Microbe In. 21: 701–708.

Urbanska, A., W. F. Tjallingii, A.F.G. Dixon, and B. Leszcynski. 1998. Phenol oxidising enzymes in the grain aphid's saliva. Entomol. Exp. Appl. 86: 197–203.

Will, T., W. F. Tjallingii, A. Thönnessen, and A.J.E. van Bel. 2007. Molecular sabotage of plant defense by aphid saliva. Proc. Natl. Acad. Sci. U.S.A. 104: 10536–10541.

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