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ALFALFA CYCLITOLS IN THE HONEYDEW OF AN APHID

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Key Word Index—*Medicago sativa* cv Caliverde; Leguminosae; alfalfa; cyclitols; *myo*-inositol; ononitol; pinitol; *Acyrthosiphon pisum*; pea aphid.

Abstract—The free cyclitols pinitol, ononitol and *myo*-inositol occur in the honeydew (excreta) of pea aphids (*Acyrthosiphon pisum*) which feed on pea aphid-susceptible alfalfa (*Medicago sativa* cv Caliverde). These cyclitols also occur in the leaves and stems of alfalfa. Aphids were incapable of *de novo* synthesis of these cyclitols. Honeydew production by the pea aphid results from ingesting phloem-sap, so the occurrence of cyclitols in honeydew results from their translocation in the phloem. The relatively high content of *myo*-inositol in honeydew indicates that it is selectively translocated. The most abundant alfalfa cyclitol, pinitol, had no effect on aphid feeding behavior at concentrations up to 1% (w/v; artificial diet).

INTRODUCTION

Aphid honeydew (excreta) frequently contains constituents, such as sugars and amino acids [1], sterols [2], various lipids [3], and phenols [4], which are translocated in the phloem of the host-plant. Phloem-sap is the diet of many species of aphids and respective translocated compounds affect aphid feeding behavior and/or nutrition [5].

Cyclitols are widely distributed throughout plants [6]. Glucinol [*O*-β-D-glucopyranosyl-(1-1)-*myo*-inositol] is found in the vascular tissue of many plants [7]. *Myo*-inositol is commonly found in plants and animals as the free cyclitol, phosphoric-ester (phytic acid), glycoside, or as a component of phospholipids [8]. Alfalfa additionally contains the cyclitols pinitol (1D-3-*O*-methyl-*chiro*-inositol) [9] and ononitol (1D-4-*O*-methyl-*myo*-inositol) [10].

The location of free cyclitols in plant tissues is not known and their biological significance to either plant or herbivore is only partially understood. *Myo*-inositol is an essential growth factor for micro-organisms, certain plant and animal cells [8], and is included in the holidic diets of most aphids [11]. *Myo*-inositol is converted into *scyllo*-inositol in the hemolymph of many species or orthopteran insects [12]. Various *O*-methyl ethers of cyclitols have been reported to have biological activities with respect to herbivorous chewing-insects. Pinitol, most frequently associated with Pinaceae, Leguminosae and Caryophyllaceae [6], was reported to have antigrowth activity against *Heliothis zea* (Boddie) [13], but recent results do not support this claim [14]. It is a phagostimulant to *Eurema hecabe mandarina* De L'Orza, whose host-plants all contain pinitol [15]. Quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) is a phagostimulant to larvae of a noctuid, *Serrodes partita* (F.), which feeds on the quebrachitol-containing leaves of *Pappea capensis* E. and Z. (Sapindaceae) [16].

This study determines if the pea aphid, *Acyrthosiphon pisum* (Harris), a legume-specific aphid, contacts and ingests cyclitols from its host-plant *in vivo*, and determines the effect of these cyclitols on the feeding behavior of the aphid.

RESULTS AND DISCUSSION

Three cyclitols, *myo*-inositol, pinitol and ononitol, were identified in the honeydew of pea aphids that fed on pea aphid-susceptible alfalfa (*Medicago sativa* L. cv Caliverde) (Table 1). Fructose, glucose, sucrose, trehalose and melezitose were also identified. The same cyclitols and sugars, except melezitose, were identified in the alfalfa. The cyclitol concentration (% fr. wt) in the above-ground parts of alfalfa were pinitol 0.38, ononitol 0.08 and *myo*-inositol 0.04. Aphids, when reared on artificial diets, could

Table 1. Relative % and ratio to sucrose of free cyclitols in the honeydew of the pea aphid (*Acyrthosiphon pisum*) and in the host-plant, alfalfa (cv Caliverde)*

Compound	Pea aphid honeydew		Alfalfa (stems and leaves)	
	Relative %	Cyclitol : sucrose	Relative %	Cyclitol : sucrose
Pinitol	41.1	0.22	74.8	1.82
Ononitol	3.0	0.07	16.6	0.40
Myo-inositol	55.9	0.27	8.6	0.21

*Quantitation based on integration of GC peak areas.

not synthesize or interconvert the cyclitols. The relative amount of pinitol and *myo*-inositol added to artificial diets was similar in honeydew excreted by aphids which fed on the diets, thus showing there was no selective absorption or excretion of these cyclitols by the aphids. Electronic monitoring of the feeding behavior of the aphids [17] on alfalfa showed that these aphids fed from the phloem and that honeydew excretion was concomitant with phloem-sap ingestion. Hence, the presence of the same cyclitols in the honeydew of the aphids as those which occur in their host-plant indicates that the cyclitols are translocated in the phloem. The relative amounts of cyclitols and sucrose in the alfalfa did not change grossly between the 24 hr of honeydew collection. However, the *myo*-inositol in honeydew was 55.9% of the cyclitol content, whereas *myo*-inositol constituted only 8.6% of alfalfa cyclitols (Table 1). Furthermore, the ratios of *myo*-inositol to sucrose in the honeydew and alfalfa were similar, while the ratios of pinitol and ononitol to sucrose in the honeydew were only a fifth as great as in the plant (Table 1). Hence, it appears there is selective phloem-loading and translocation of *myo*-inositol over the other cyclitols. This selective translocation may reflect the significance of *myo*-inositol in plant biosyntheses of cell wall polysaccharides, certain hormones (e.g. IAA esters, phytic acid) and other cyclitols from hexoses [18]. We found *myo*-inositol in the honeydew of two other species of aphids whose host-plants do not contain other cyclitols: *Aphis gossypii* Glover fed on cucumber (*Cucumis sativus* L.) and *Schizaphis graminum* (Rondani) fed on sorghum [*Sorghum bicolor* (L.) Moench]. *Myo*-inositol has also been identified in the sap of various trees [19, 20].

The most abundant cyclitol in alfalfa, pinitol, has no discernible impact on the feeding behavior of the pea aphid. Artificial diets supplemented with up to 1% pinitol (twice the concentration in the host-plant) were fed on by the aphids equivalently to control diets. Since the alfalfa cultivar resistant to pea aphid, CUF-101, contains only a third as much pinitol as cv Caliverde, the concentration of pinitol in alfalfa does not seem to be correlated with the resistance of alfalfa to pea aphids.

EXPERIMENTAL

Identification of cyclitols. Honeydew was eluted with warm H_2O from the surface of Al foil placed for 24 hr beneath aphid-infested plants. The eluant was filtered, diluted with EtOH to 80% aq. EtOH, refiltered and concd *in vacuo*. Honeydew from aphids reared on artificial diets was handled similarly. After the aphids were dislodged, stems and leaves of alfalfa plants were freeze-dried, ground, extracted with hexane and then with aq. MeOH. The aq. MeOH extract was diluted with H_2O, treated with decolorizing carbon, filtered through Celite and concd *in vacuo*. Conc. honeydew and alfalfa extracts were next treated with anion exchange resin (Dowex 3), filtered and freeze-dried. TMSi ether derivatives were prepared by treating the residues with HMDS and TMCS (2:1) in pyridine. After the removal of pyridine, TMSi ether derivatives were taken-up in *iso*-octane and cyclitol-TMSi compounds were identified by comparing GC RR_f (fructose-TMSi = 1.00) to authentic samples on 3% OV-17 and 3% OV-101, respectively: pinitol-TMSi 1.04, 1.03; ononitol-TMSi 1.31, 1.32; *myo*-inositol-TMSi 1.53, 1.66. MS of the isolated cyclitol-TMSi ethers were compared to those of authentic compounds [21]. MS (GC) 70 eV *m/z* (rel. int.): pinitol-TMSi: 539 $[M-Me]^+$ (1), 318 (32), 260 (44), 217 (40), 147 (33) and 73 (100); ononitol-TMSi: 507 $[M-Me$ and $-MeOH]^+$ (1), 318 (16), 217 (50), 191 (26), 147 (29) and 73 (100); *myo*-inositol-TMSi: 612 $[M]^+$ (1), 342 (6), 318 (27), 305 (48), 217 (63) and 73 (100). Sugar-TMSi ether derivatives were similarly identified by GC, then had their identity confirmed by GC/MS (except melezitose, which is a common trisaccharide in aphid honeydew [22]).

Aphid feeding behavior and artificial diets. The feeding behavior of pea aphids on greenhouse-grown alfalfa was monitored electronically according to methods previously outlined [17, 23]. Aphids were transferred from plants to artificial diets [24] with or without 0.05% (w/v) *myo*-inositol or 1.0% pinitol and allowed to feed for 24 hr. The aphids were then transferred to fresh test diets after which honeydew was collected for analysis.

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