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Article Addendum

Contribution of Glucosinolate Transport to Arabidopsis Defense Responses

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Addendum to:

Myzus persicae *(Green Peach Aphid) Feeding on* Arabidopsis *Induces the Formation of a Deterrent Indole Glucosinolate*

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ABSTRACT

Accumulation of glucosinolates, a class of defense-related secondary metabolites found almost exclusively in the Capparales, is induced in response to a variety of biological stresses. It is often assumed that elevated glucosinolate levels result from de novo biosynthesis, but glucosinolate transport from other parts of the plant to the site of herbivory or pathogen infection can also contribute to the defense response. Several studies with Arabidopsis and other crucifers have demonstrated that glucosinolates from vegetative tissue are transported to developing seeds. Here we discuss evidence that long-chain aliphatic glucosinolates are transported to the site of herbivory in response to *Myzus persicae* (green peach aphid) feeding on Arabidopsis.

INTRODUCTION

Although analysis of Arabidopsis gene expression[1] shows that glucosinolate biosynthesis occurs in most or all plant organs, the phloem mobility of these metabolites[2] suggests that transport within cruciferous plants also contributes to the overall glucosinolate profile. In particular, several lines of evidence prove that glucosinolates are transported from vegetative tissue to developing seeds. In reciprocal crosses between lines with differing glucosinolate profiles, the seed glucosinolate content of *Brassica napus* and Arabidopsis is influenced by the maternal genotype.[3,4] Isotopic labeling experiments with *Sinapis alba* showed that, although the majority of seed glucosinolates are synthesized in the silique walls, at least some glucosinolate biosynthesis occurs in the seeds themselves, which contain all of the necessary enzymes.[5] Glucosinolate transport between plant organs has been demonstrated in Arabidopsis through the application of radiolabeled *p*-hydroxybenzylglucosinolate to the leaves and subsequent detection in the seeds.[6]

LONG-CHAIN ALIPHATIC GLUCOSINOLATES ACCUMULATE AT THE SITE OF APHID FEEDING

Recent research in our laboratory[7] showed that, in response to *Myzus persicae* (green peach aphid) feeding on Arabidopsis, indol-3-ylmethylglucosinolate is converted into the more aphid-deterrent 4-methoxyindol-3-ylmethylglucosinolate (4MI3M). When aphids are feeding on whole plants or detached leaves, 4MI3M is the only glucosinolate that shows increased accumulation. However, when aphids are caged on an individual Arabidopsis leaf, long-chain aliphatic glucosinolates, in particular 8-methylsulfinlyloctylglucosinolate (8MSO), accumulate in the aphid-fed leaves to almost twice the level of leaves with aphid-free control cages. A likely, though as yet unproven, explanation for these observations is that 8MSO is transported from elsewhere in the plant to the site of aphid feeding. No significant changes in 8MSO levels were observed in systemic leaves of the aphid-fed plants, but this is perhaps not surprising because the glucosinolate assay is rather variable and relatively small amounts of transport from the remaining leaves of the plant could cause a significant increase in a single aphid-fed leaf. Further evidence for transport comes from the observation that, in comparison to other glucosinolates, the concentration of 8MSO in aphid honeydew is two to three-fold higher than in the whole plant and in phloem sap that was released after ethylene diamine tetraacetate (EDTA) treatment[8] of Arabidopsis rosettes that were cut off at soil level (Fig. 1A).

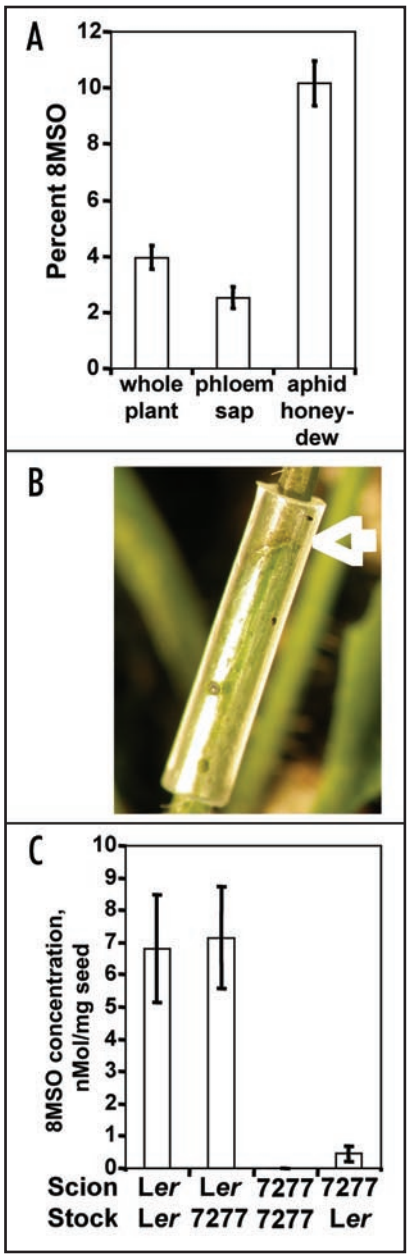

Figure 1. (A) 8-methylsulfinyloctylglucosinolate (8MSO) content as a percentage of total glucosinolates in the whole plant, phloem sap, and aphid honeydew. Mean ± st. dev. (B) Close-up view of an Arabidopsis flower stalk graft with a plastic sleeve holding the stock and scion together. The white arrow indicates the graft junction. (C) Seed 8MSO content (nMol/mg) in reciprocally grafted Ler wild type and line 7277 mutant plants. Mean ± st. dev. of N = 6 to 9.

LONG-CHAIN ALIPHATIC GLUCOSINOLATES ARE TRANSPORTED FROM LEAVES TO SEEDS

We used Arabidopsis flower stalk grafting[9] (Fig. 1B) and seed glucosinolate assays[4] to show that endogenous long-chain aliphatic glucosinolates can be transported between plant organs. Landsberg *erecta* (L*er*) wild type and line 7277, an L*er mam-L* mutant that is almost completely devoid of long-chain aliphatic glucosinolates,[4,10] were used for reciprocal grafting experiments. Seed glucosinolate profiles from ungrafted L*er* and an L*er*-L*er* self-graft were not significantly different (data not shown), indicating that the graft junction itself does not affect glucosinolate accumulation. Seven independent isolates of line 7277 grafted onto itself had undetectable amounts of 8MSO in the seeds. However, when 7277 was grafted as a scion onto wild-type L*er*, the 8MSO accumulation was increased from undetectable levels to 0.5 nMol/mg seed (Fig. 1C), showing that there is at least some transport of 8MSO from the rosette leaves to the developing seeds. Other long-chain aliphatic glucosinolates were also elevated in the seeds when line 7277 was grafted onto L*er* wild type (data not shown). 8MSO accumulation in seeds of self-grafted L*er* and L*er* grafted onto line 7277 was not significantly different (Fig. 1C; 6.8 ± 1.7 vs. 7.1 ± 1.6 nMol/mg; $p > 0.05$, Student's t-test). This suggests that the majority of seed 8MSO is synthesized in the siliques or the seeds themselves.

FUTURE PROSPECTS FOR RESEARCH ON DEFENSE-RELATED GLUCOSINOLATE TRANSPORT

Although most glucosinolate biosynthetic enzymes in Arabidopsis have been identified,[11] almost nothing is known about the molecular biology of glucosinolate transport. Given the numerous and readily available Arabidopsis biosynthetic mutants, grafting experiments similar to those in Figure 1 will allow investigation of induced glucosinolate transport in response to herbivory. Alternate grafting methods, whereby two plants share a common rootstock[12] or the graft junction is within the leaf rosette,[13] will facilitate analysis of root-to-shoot transport and transport between different leaves on the same plant. Glucosinolate transport proteins are likely to exist in the cell membranes of Arabidopsis and other crucifers, but they have not yet been identified. Given the strong dependence of seed glucosinolate content on maternal genotype, a selection for Arabidopsis mutants with altered seed glucosinolate accumulation might allow the identification of these glucosinolate transporters. Once such transporters have been identified, analysis of knockout mutants will determine whether these or related proteins are also involved in defense-related glucosinolate transport and accumulation.

References

1. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W. Genevestigator: Arabidopsis microarray database and analysis toolbox. Plant Physiol 2004; 136:2621-32.
2. Brudenell AJP, Griffiths H, Rossiter JT, Baker DA. The phloem mobility of glucosinolates. J Exp Bot 1999; 50:745-56.
3. Magrath R, Mithen R. Maternal effects on the expression of individual aliphatic glucosinolates in seeds and seedlings of *Brassica napus*. Plant Breeding 1993; 111:249-52.
4. Kliebenstein DJ, D'Auria JC, Behere AS, Kim JH, Gunderson KL, Breen JN, Lee G, Gershenzon J, Last RL, Jander G. Characterization of seed-specific benzoyloxyglucosinolate mutations in *Arabidopsis thaliana*. Plant J 2007; In press.
5. Du L, Halkier BA. Biosynthesis of glucosinolates in the developing silique walls and seeds of *Sinapis alba*. Phytochemistry 1998; 48:1145-50.
6. Chen S, Petersen BL, Olsen CE, Schulz A, Halkier BA. Long-distance phloem transport of glucosinolates in Arabidopsis. Plant Physiol 2001; 127:194-201.
7. Kim JH, Jander G. *Myzus persicae* (green peach aphid) feeding on Arabidopsis induces the formation of a deterrent indole glucosinolate. Plant Journal 2007; 49:1008-19.
8. King RW, Zeevaart JAD. Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol 1974; 53:96-103.
9. Rhee SY, Somerville CR. Flat-surface grafting in *Arabidopsis thaliana*. Weeds World 1995; 2:8-11.
10. Textor S, de Kraker J, Hause B, Gershenzon J, Tokuhisa JG. MAM3 catalyzes the formation of all aliphatic glucosinolate chain lengths in *Arabidopsis thaliana*. Plant Physiol 2007; 144:60-71.
11. Halkier BA, Gershenzon J. Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 2006; 57:303-33.
12. Turnbull CG, Booker JP, Leyser HM. Micrografting techniques for testing long-distance signalling in Arabidopsis. Plant J 2002; 32:255-62.
13. Ayre BG, Turgeon R. Graft transmission of a floral stimulant derived from constans. Plant Physiol 2004; 135:2271-8.