Effects of secondary compounds on host plant preference by the phloem-feeding insect, *Bemisia tabaci* and predatory omnivorous insect, *Orius laevigatus*.

Thesis

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By

Dinesh Kafle

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ABSTRACT

Phytophagous insects have developed intimate relationships with their host plants. To reduce insect attack, a wide-range of defense mechanisms has evolved in plants. These defenses primarily involve the chemical and physical characteristics of the plants. In parallel, insects develop multiple behavioral and physiological traits that allow them to overcome plant defenses while feeding, developing and reproducing on defended host plants. Moreover, the long exposure to insects has supposedly played a major role in plant speciation that resulted in high taxonomic diversity of plants. To protect themselves from attack, plants have evolved both constitutive and induced defenses. Constitutive defenses include physical barriers that impede pathogen or insect access to plant tissues, as well as pre-formed chemical defenses (secondary metabolites) that have antixenotic or antibiotic effects. Induced plant defense mechanisms are activated upon tissue damage and include the synthesis of various defensive compounds. Volatile compounds emitted by plants play key role in the plant defense system and also act as attractant for many natural enemies of herbivorous insects.

In plants of the order Capparales, which includes cruciferous crops and *Arabidopsis thaliana* plants, the glucosinolates–myrosinase system serves as a major chemical defense mechanism against insects, bacteria and fungi. Glucosinolates (β-thioglucoside-N-hydroxysulfates) are amino acid-derived secondary metabolites that can be cleaved by the enzyme myrosinase (β-thioglucoside glucohydrolase). Myrosinase and glucosinolates are physically separated in undamaged tissue. In *A. thaliana*, glucosinolates (GSs) are primarily stored in cells adjacent to the vascular bundles, and myrosinases are stored in specialized idioblasts in the phloem. When cellular disruption occurs during herbivore attack, GSs come in contact with myrosinases, resulting in the production of a variety of toxic degradation products, including isothiocyanates, nitriles, thiocyanates, oxazolidine-2-thiones and epithionitriles.

The tobacco whitefly, *Bemisia tabaci* (Gennadius) is a polyphagous, phloem-feeding insect which causes serious agricultural yield losses both quantitatively and qualitatively. It damages the plants by manipulating growth, depleting photoassimilates, transmitting viruses and even injecting toxins into the plant. Unlike chewing insects, such as caterpillars and beetles, *B. tabaci* does not cause extensive cellular disruption, Instead, *B. tabaci* probes plant tissue intercellularly to establish feeding sites in the phloem sieve elements; feeding at these sites may be maintained for hours or even weeks. This mode of feeding minimizes wounding and limits the local induction of defense response. *Orius laevigatus* (Fieber) is an omnivorous predatory bug that is being used as a biological control agent against several insect pests such the Western Flower Thrips, *Frankliniella occidentalis. O. laevigatus* are equipped with piercing-sucking type of mouthparts. They feed on the live preys such as thrips, aphids, whiteflies and eggs of moth. In addition to live prey, they feed on pollen and plant sap from xylem and mesophyll.

In the present research, I quantified changes in the behavior and performance of *B. tabaci* 'biotype B' and *O. laevigatus* in response to elevated level of glucosinolates in *A. thaliana* plants (ecotype Columbia) over-expressing the transcription factor MYB51. MYB51 was overexpressed using both constitutive (35S) and phloem-specific (pSUC) promoters. These transgenic plants contained significantly higher levels of Tryptophan-derived indolic glucosinolates. Homogeneous and heterogeneous choice experiments were carried out using wild-type (WT) and MYB51-35S plant genotypes that were offered to *B. tabaci* in homogeneous (WT / WT, MYB51-35S / MYB51-35S) or heterogeneous (WT / MYB51-35S) arenas. One newly emerged female was introduced into each arena, and its behavior was recorded with a digital camera for 5 days. The oviposition behavior and performance *O. laevigatus* were tested in Homogeneous and heterogeneous choice treatments using the plant genotypes WT, MYB51-35S and MYB51-pSUC.

Elevated indolic-glucosinolate levels affected *B. tabaci* negatively mostly in the heterogeneous plant settings. Upon eclosion, it took the adult *B. tabaci* significantly longer to select a feeding site in the heterogeneous than homogeneous environments. In addition, *B. tabaci* females deposited significantly fewer eggs on both WT and MYB51-35S leaves in the heterogeneous environments. Also, *B. tabaci* females in heterogeneous environments remain longer on WT leaves than on MYB51-35S leaves. *B. tabaci* seemed to be more restless in heterogeneous environments because the rate of switching between experimental leaves and between leaves and the arena walls was higher compared to those in the homogenous environment. The rate of movements within MYB51-35S leaves in heterogeneous treatment was significantly higher than that for WT.

The oviposition behaviors (total egg deposition and oviposition site selection) of the predatory omnivore *O. laevigatus* were affected only when offered *A. thaliana* plants with higher levels of pSUC-driven indolic glucosinolates. Female oviposition was similar in all homogenous host environments (WT, 35S and pSUC). In the heterogeneous WT/ MYB51-pSUC and MYB51-35S/ MYB51-pSUC environments, female bugs preferentially oviposited on WT or MYB51-35S plants over pSUC plants. The phloem specific elevation of indolic glucosinolates had also been found to affect the oviposition site selection of *O. laevigatus*. They were found to lay their eggs equally on both sides of the leaves in the MYB51-pSUC leaves, while on other two plant genotypes, they were found to lay more than 80% eggs on abaxial side.

The mixed glucosinolate specific volatiles in the heterogeneous environments may have inhibited settling and oviposition in *B. tabaci*. The simple nervous system of many polyphagous insect herbivores, such as whitefly and aphids, may limit their ability to process multiple sensory information in mixed host environments, and effectively identify host plants suitable for feeding and oviposition. The distraction *B. tabaci* experienced in the mixed host systems may be attributed to the complex sensory stimuli that may have reduced its overall performance in these environments. The present study suggests that *O. laevigatus* had either detoxified or avoided the glucosinolates because it was hardly affected by the high levels of glucosinolates.

In conclusion, the mixed host environments that comprised of plants with various levels of secondary compounds were found to adversely affect the polyphagous herbivore *B. tabaci*. In contrast, the higher levels of pSUC-driven glucosinolates were found to exert some negative effect on the omnivorous bug *O. laevigatus.* These results that were obtained for Arabidopsis plants may also be applicable to economically important cruciferous crops. If so, mixed cropping systems may be expected to harbor lower herbivore populations compared to monocultures, with little adverse affects on natural enemies.