The Chemical Mechanism for Defense of Glandular-Haired Alfalfa against the Potato Leafhopper

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The potato leafhopper, *Empoasca fabae* (Harris), is considered the most significant pest of alfalfa, *Medicago sativa* L., in the Midwestern and Eastern United States. Commercial varieties of glandular-haired alfalfa are currently available with resistance to this key forage pest. Unique to the resistant varieties is the presence of glandular trichomes mainly on the stems and petioles. Previous studies have indicated that biologically-active compounds localized in the glandular trichomes provide a chemically-based form of resistance. In order to improve resistance levels and management decisions, bioassay-guided fractionations were performed to identify the trichome metabolites responsible for leafhopper resistance.

Crude glandular trichome extracts were prepared from the proprietary glandular-haired genotype *M. sativa* G98A (Cal/West Seeds), along with nonglandular trichome extracts from the susceptible cultivar Ranger. Two-choice bioassays determined crude G98A glandular trichome extracts exhibited dose-dependent deterrence to leafhopper settling. Subsequent fractionations isolated an individual fraction with strong, dose-dependent deterrence to leafhopper settling. Large, nonvolatile fatty acid amides, $C_nH_{2n+1}NO$ (n = 19–23), were major components of the active fraction, and these compounds were absent in trichome extracts from the susceptible cultivar Ranger. Mass spectrometry identified the unique trichome metabolites as *N*-(3-methylbutyl)- amides and *N*-(methylpropyl)amides of $C_{14:0}$ through $C_{18:0}$ fatty acids, along with the *N*-(3-methylbutyl)amide of linoleic acid ($C_{18:2}$). Following their isolation and purification, the fatty acid amides unique to G98A maintained their deterrence to leafhopper settling. Supplementation of the purified amides with authentic free fatty acids resulted in increased activity, but the authentic free fatty acids alone were not deterrent. These results indicated the unique nonvolatile fatty acid amides were primarily responsible for settling deterrence. Furthermore, when tested singly, the synthetic form of *N*-(3-methylbutyl)amide of linoleic acid exhibited dose-dependent deterrence.

Since previous studies indicated volatiles from glandular-haired alfalfa might also be playing a role in resistance (along with the nonvolatile trichome metabolites), stem, leaf, and whole plant volatiles were collected from G98A and Ranger. When given a choice, adult leafhoppers oriented towards leaf and whole-plant volatiles collected from Ranger over G98A. While no volatiles were unique to G98A or Ranger, different ratios of the same volatiles were detected. Instead of producing volatile repellents, leafhoppers appear to be less attracted towards the glandular-haired G98A.

Overall, this project provides critical insight into the chemical basis for resistance of glandular-haired alfalfa against the potato leafhopper. Nonvolatile fatty acid amides secreted by the glandular trichomes act as the primary contributor to leafhopper resistance, which is based on behavioral deterrence. Resistance levels of glandular-haired alfalfa could likely be improved by selecting for plants producing higher amounts of the fatty acid amides identified in this study. As such, additional studies are needed to understand the genetic basis for the production and localization of the fatty acid amides. While the blend of volatiles produced by G98A appears to lead to decreased attraction, breeding for the production of lower levels of attractive volatiles is not likely to be an effective strategy under a monoculture setting.