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Role of Glycine and GABA Secreted in the Digestive Juice of Privet-Specialist Insects: Counteraction of Herbivorous Insects Against Chemical Defense of Host Plant

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High concentrations (0.4-1.5 %) of free glycine existed in the digestive juice of several Lepidoptera and Hymenoptera larvae that feed on the privet tree, *Ligustrum obtusifolium* (Oleaceae), while in the digestive juice of non-privet feeders, concentrations of glycine was very low. Injection experiments using ^{15}N-labeled amino acids showed that glycine was secreted in midgut lumen from hemolymph in an active and selective manner in a privet-specialist species, *Brahmaea wallichii* (Brahmaeidae), which had 50 mM of free glycine in the digestive juice. Leaves of the privet tree contain oleuropein (3% per wet weight), a phenolic iridoidglycoside, probably as a defense chemical. When the leaf cells are damaged, β-glucosidase and polyphenol oxidase that are kept in the leaves separately from oleuropein activate it into a very strong protein-denaturant. When, in particular, β-glucosidase deglucosidate the iridoid-glycoside moiety of the oleuropein molecule, this moiety is converted into glutaraldehyde-like structure and crosslinks protein molecules to form high molecular weight complexes and makes protein non-nutritive by decreasing the lysine content. *In vitro* experiments showed that 1% glycine could completely inhibit the activities of glycine. Further *in vivo* experiment using the Eri silkworm (*Samia ricini*) showed that glycine can neutralize the adverse effect of the privet leaves. These results suggested that free glycine is secreted in the digestive juice as a counteraction to chemical defense of the host plant. Our data indicated that the amino residue in free glycine is responsible for the inhibition. We also found that, in some Lepidoptera species, such as *Artopoetes pryeri* (Lycaenidae), high concentrations of other amino acids such as GABA and β-alanine existed in the digestive juice, which seem to play the same role as glycine does. Interestingly, amino acids found in the privet specialist species, such as glycine, GABA and β-alanine showed strong inhibition to the denaturing activity of the privet leaves in lower concentrations, while amino acids such as alanine, which was not found in the digestive juice of any of the

privet specialists, showed weak inhibition in higher concentrations. Our results are clear and interesting examples of both coevolution of plants and herbivores mediated by chemicals and convergent evolution of insect counteraction.