Regulation of grasshopper fecundity, longevity and egg viability by plant growth hormones[1]

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Summary. Abscisic acid, a plant growth hormone, added in water to the grass diet of a grasshopper, greatly reduces the reproductive rate, as does gibberellin A_3 at doses 10 times greater. Reciprocal seasonal variations in quantities of these hormones could synchronize the growth and reproduction of insects with their host plants in the field.

The reproductive physiology of univoltine phytophagous insects is seasonally correlated with the occurrence of their host plants. Changing environmental conditions may affect plant growth and alter plant metabolites, but little is known about how such changes influence the physiology of insects feeding on them. The growth temperature or other environmental factors affecting western wheatgrass, *Agropyron smithii* Rydb., the primary host plant of the rangeland grasshopper *Aulocara elliotti* (Thomas), significantly affects the fecundity, egg viability and female longevity of that insect independent of its own rearing environment[2]. Since seasonal changes are known to affect plant hormone concentrations[3], tests were carried out to determine whether such hormonal changes might underlie these reproductive responses. The results indicate that 2 plant hormones, abscisic acid (ABA) and gibberellin A_3 (GA_3), added to the wheatgrass diet, significantly alter fecundity and egg viability of *A. elliotti*. The biological effects of crude lipid extracts of wheatgrass, as well as juvenile hormone (JH III), a grasshopper vitellogenic hormone[4], were also tested for comparison.

Materials and methods. Wild *A. elliotti* collected as 5th instar nymphs or newly moulted adults, were reared in pairs with natural daylengths and diurnally fluctuating temperatures (24–30 °C) according to published methods[5]. Western wheatgrass sod transplanted from a field site was grown in the greenhouse with natural daylengths and diurnally fluctuating temperatures (mean max. 33 °C; mean min. 17 °C; range, 41–11 °C) during the experiment, June 25–September 15. 30 pairs of adults were allotted to each of 8 treatment regimens: GA_3 at 60 and 600 mg/l distilled water (Grade III, No. G3250); ABA at 6 and 60 mg/l distilled water (Mixed isomers, No. A7383) both from Sigma Chemical Co., St. Louis); JH III (Calbiochem Inc., San Diego) at 0.5 and 5.0 ml/l distilled water (all hormones dissolved in 10 ml of ethanol before dilution); chloroform-methanol extracts[6] of 30 g of wheatgrass (WGE) in 1 liter distilled water, thought to contain lipoid hormones, and untreated wheatgrass controls watered with distilled water. Cut grass, 2–3 g/food vial, was soaked in and watered with the treatments solutions while being fed.

Results and discussion. ABA at 6 and 60 mg/l and GA_3 at 60 and 600 mg/l distilled water significantly reduced the fecundity, egg viability and rate of reproduction (numbers of eggs per adult day) of *A. elliotti*. JH III at 0.5 mg/l reduced fecundity similarly to that of GA_3 at 600 mg/l. The reproduction of grasshoppers fed WGE resembled that of those fed JH III at the 5.0 ml/l dose. Female longevity was decreased significantly only by WGE.

Reduced reproduction effected by low concentrations of ABA and GA_3 added to the host grass may mean that these compounds act on the insect's metabolism via its hormonal pathways. ABA, GA_3 and JH III are biochemically similar terpenoid compounds derived from mevalonate[7–9]. ABA and JH III are sesquiterpenoids derived from farnesol[7,9], while GA_3 is a diterpenoid derived from geranylgeraniol[8], and ecdysone, a sterol derivative[10], may be similarly derived via a monoterpenoid pathway. The closer relationship of ABA to JH III may explain why similar reproductive effects are exhibited at 10-fold higher concentrations of GA_3. Ecdysones stimulate or inhibit growth in *Drosophila* cells depending on concentration, while JH counteracts both effects[11]. ABA resembles recently discovered precocenes, anti-JH compounds which sterilize certain insects[12]. Retarded growth and sexual maturation in locusts fed senescent vegetation was reversed by adding GA_3[13,14], yet GA_3 reduced fecundity in other insects[15,16].

ABA caused weak JH effects when fed to mealworms[17] and pyrrhocorid bugs[18], but stimulated fecundity and rate of development in *Aphis fabae* fed on leaf discs[19]. Since larger doses of ABA must be applied to leaf discs than to intact plants to get similar senescent effects[6], growth stimulation in *A. fabae* by ABA could be related to concentration effects associated with leaf disc techniques. ABA added to intact barley leaves reduced fecundity in the aphid *Rhopalosiphum padi* L.[20]. Concentrations of ABA increase as daylengths decrease[3] to bring about plant senescence, while GA_3, abundant in young plants, may be deficient in aging plants[12]. Likewise, plant sterols vary in concentration seasonally[21]. Since the metabolism of endogenous as well as applied plant hormones in western wheatgrass is unknown, how these might interact to affect grasshopper reproduction is uncertain. When these plant-insect interactions are understood, it should be possible to use ABA as a safe control agent for locusts and other phytophagous insects. Research with many phytophagous species will be required to assess

The effects of exogenous dietary hormones and grass extract upon fecundity, egg viability, and longevity in the grasshopper *Aulocara elliotti* (Thomas)

Treatment regimen	No. of fertile pairs	Mean No. of eggs per female	Mean No. of viable eggs per female	Eggs laid per adult and day	Mean longevity of fertile female
Western wheatgrass (control)	21	24.7	14.3	0.674	36.6
GA_3 - 60 mg/l distilled water	13	11.7**	1.3***	0.278***	42.0
GA_3 - 600 ml/l distilled water	25	18.6**	8.3**	0.439**	42.4
ABA - 6 mg/l distilled water	13	10.6***	1.5***	0.254***	41.7
ABA - 60 mg/l distilled water	17	14.5**	6.7***	0.436**	33.4
Wheatgrass extract (WGE)	17	24.1*	10.2*	0.785	30.7*
JH III - 0.5 ml/l distilled water	21	17.7**	5.8***	0.479*	36.9
JH III - 5.0 ml/l distilled water	23	21.5*	9.9*	0.599	36.0

Statistical comparisons are between treatment regimens and the control. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

the validity of the hypothesis that plant hormones serve as biochemical signals to regulate insect reproduction seasonally.

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Uptake of ^{3}H-GABA (γ-aminobutyric acid) and ^{3}H-leucine in the pancreatic islets and substantia nigra of the rat

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Summary. Isolated pancreatic islets and thin slices of substantia nigra (SN) of the rat were incubated in a medium containing ^{3}H-GABA or ^{3}H-leucine to test the activity of both tissues in the uptake of those substances. Pancreatic islets showed a low uptake of both ^{3}H-GABA and ^{3}H-leucine, but SN had a high activity in the uptake of ^{3}H-GABA, though not for ^{3}H-leucine. This suggests that GABA contained at high levels in the pancreatic islets plays some functional role other than in neurotransmission as in the central nervous system (CNS).

γ-Aminobutyric acid (GABA) and its synthesizing enzyme, glutamate decarboxylase (GAD), have been mainly found in the invertebrate and vertebrate nervous system, where GABA functions as an inhibitory neurotransmitter[3, 4]. On the other hand, GABA and GAD have also been found in non-neuronal tissues such as kidney, liver, blood vessel, pancreas and pancreatic islets, although at much lower concentrations than in nerve tissue[5–8]. In the previous study from our laboratory, as high a concentration of GABA and as high a GAD activity as in the central nervous system (CNS) were found in rat pancreatic islets and in human insulinoma[9, 10]. In brain tissue a high level of uptake of ^{3}H-GABA into glial cells and nerve terminals has been reported, and this uptake is considered to be important for the inactivation and reutilization of GABA as the neurotransmitter[11, 12]. In this respect it seems worthwhile to investigate whether or not GABA in the pancreatic islets functions in the same manner as in the nervous system. In this paper the uptake of ^{3}H-GABA and ^{3}H-leucine in isolated pancreatic islets was studied in comparison with that of a thin slice of substantia nigra (SN) which contains the highest amount of GABA in the CNS[4].

Materials and methods. Albino Wistar rats (200–300 g) were anesthetized with pentobarbiton sodium (50 mg/ml/kg) and the abdominal cavity was opened. The pancreas was carefully excised after the injection of 10 ml of Krebs Ringer solution containing collagenase (25 mg/10 ml, Boehringer Mannheim) into the pancreas through the choledox duct. The pancreas was chopped and incubated at 37 °C with mechanical stirring to separate the islets from exocrine gland. This isolation of the islets was performed with a minor modification, according to the method of Lacy and Kostianovsky[13]. The skull was opened and the brain was removed under the same anesthesia. Using a glass guide and a razor blade a thin section (400 µm in thickness) of SN was prepared[14] and the weight was determined with a torsion balance. After preincubation of the isolated islets and the SN slice for 20 min in 0.5 ml of standard medium (concentration in mM: NaCl 125, KCl 5, KH_2PO_4 1.24, $MgSO_3$ 1.3, $NaHCO_3$ 26, $CaCl_2$ 1.3, glucose for pancreatic islets 3.3 and for SN slice 8.0), stirred and kept under a 95% O_2 and CO_2 atmosphere, the tissues were further incubated for 50 min in the standard medium containing radioactive substances for the uptake study. For the autoradiographic study, the pancreas tissue was chopped into pieces (0.5–1 mm^2) using a razor blade. The chopped pancreas tissue and the SN slice were incubated together in the same medium in the manner described above. The temperature of the medium was kept at 37 °C throughout the experiment.

Results and discussion. Thin slices of SN and isolated pancreatic islets were incubated in the medium containing ^{3}H-GABA or ^{3}H-leucine. As indicated in the table, the SN slice showed a high activity in the uptake of ^{3}H-GABA with a high uptake ratio, whereas the pancreatic islets showed very low uptake. The high uptake of ^{3}H-GABA in SN confirmed the results of previous studies[14–16]. In contrast to the high uptake of ^{3}H-GABA by SN, both SN and pancreatic islets showed a low uptake of ^{3}H-leucine, with similar ratios of uptake, as indicated in the table.