Metabolism of Ingested Auxins in the Bug *Lygus disponi*:
Indole Compounds Appearing in Excreta of Bugs Fed
with Host Plants and the Effect of Indole-3-Acetic
Acid on the Feeding^{1,2}

Kōji HORI

Laboratory of Entomology, Obihiro University, Obihiro, Hokkaido 080, Japan

(Received October 1, 1979)

Several indole compounds were detected in the excreta of bugs, *Lygus disponi* LINNAVUORI, fed with rape flower bud. The Rf values on thin layer chromatogram were 0.02, 0.13, 0.18, 0.25 and 0.30 in the solvent system butanol-ethanol-water (76 : 19 : 5). These were in general accord with those of the indole-3-acetic acid (IAA) metabolites in excreta of bugs fed with IAA. Significantly high auxin activity was detected in the crude excreta of the bugs fed with various host plants, but not in the fractions of the excreta fractionated by thin layer chromatography. IAA in high concentration inhibited the feeding of the bugs, whereas IAA in suitable concentration had a tendency to stimulate it in the presence of sucrose. The significance of indole auxin metabolism in the nature is discussed.

INTRODUCTION

The bug *Lygus disponi* LINNAVUORI (Hemiptera:Miridae) as well as other mirid bugs is fond of feeding on growing tissues of host plants such as meristem, bud, flower bud, young seed, and so on (DEMPSTER, 1964; HORI, 1971; LEIGH, 1976), where auxins may be contained in higher concentration. Indole auxins seem to be toxic to organisms, so the bugs fed with them may have to detoxicate and excrete them without absorption in the haemolymph. It has been known that some insects metabolize auxins to non-toxic substances, while others utilize them for development and reproduction (SCHEURER, 1976). It has been reported in previous papers that the bug *L. disponi* fed with indole-3-acetic acid (IAA) in the laboratory converted it in the gut to some high molecular substances by combining with substances such as sugars, amino acids or proteins (HORI and ENDO, 1977; HORI, 1979). This seems to be a detoxication mechanism and a mechanism preventing the ingested IAA from being absorbed into the haemolymph (HORI, 1979). However, there has been no evidence that the bugs actually ingest indole auxins from the host plants and metabolize them in the gut to non-toxic high molecular substances to excrete the most part. The present study was performed to confirm whether or not this mechanism is also sig-

¹ Contribution No. 97 from the Laboratory of Entomology, Obihiro University.

² This work was aided in part by a grant from the Ministry of Education No. 356031.

nificant in the case of the host-plant-feeding bugs in nature and to investigate the effect of IAA on the feeding of adult bugs.

MATERIALS AND METHODS

Insects used. Adults collected from the field and starved for 24 hr before feeding were used for the experiments.

Collection of excreta. Each bug was fed respectively with rape flower buds, rape stems, clover flowers, sugar beet leaf veins, and 10% sucrose with 0.1% IAA, for control, in the feeding cages devised by MILES and HORI (1977). The host plants were cut into several pieces and put in the diet chambers. The excreta deposited on the filter paper in the feeding cages were eluted with water according to the method described by HORI (1979). The extracts were then lyophilized in the dark and kept at -18°C until use.

For the investigation of the effect of IAA on the feeding, the bugs were fed with sucrose (10%)-IAA (0.5, 0.1, 0.05 and 0.01%)-amaranth (0.01%) solution in the feeding cages. The excreta deposited on the filter paper were eluted with water and their per cent absorbancies were measured at 530 nm for estimating the amount of feeding of the bugs.

Thin layer chromatographic (TLC) analysis for indole compounds in excreta. Each of the stocked dry excreta of host-plant-feeding and control bugs was dissolved in 1.0 ml of 50% ethanol. The procedure for TLC analysis was the same as that described previously by HORI (1979). The solvent systems used are shown in Table 1.

Avena assay for auxin activity of excreta. The procedure for *Avena* assay was the same as that followed by HORI (1979).

RESULTS AND DISCUSSION

Mortality of L. disponsi fed with various diets

Although the mortality of *L. disponsi* was high when it was fed with natural host-plant tissues (Table 2), the excreta collected were sufficient for analyzing indole compounds.

Indole compounds in excreta of L. disponsi fed with natural host-plants

Only a trace of indole compounds were detected in the excreta of a few bugs fed with rape stem, clover flower and sugar beet leaf vein, whereas several indole compounds were obviously detected in the excreta of the bugs fed with rape flower bud (RFB-excreta) (Fig. 1). This difference does not always mean that the bugs ingested a larger amount of indole compounds from the latter than from each of the former, because the amount of excreta contained in the test solutions may be different among the bug groups fed with the respective host plants. In the case of solvent I, the Rf

Table 1. DEVELOPING SOLVENT SYSTEMS USED FOR TLC ANALYSIS

No.	Solvent composition (v/v)
I	Butanol-Ethanol-Water (76+19+5)
II	Chloroform-Ethanol(96%) (80+20)
III	Chloroform-Ethyl acetate-Formic acid (35+55+10)

Table 2. MORTALITY OF *L. disponsi* FED WITH VARIOUS DIETS

Diet	Duration of feeding (days)	No. of insects at the start of feeding	No. of insects after the feeding period	Mortality (%)
IAA-sucrose	2	48 ^a	40	16.7
Rape flower bud	2	49	23	53.1
Rape stem	2	51	20	60.8
Clover flower	2	51	21	58.8
Sugar beet vein	2	52	24	53.8

^a 4 replications of about 13 bugs.

Fig. 1. TLC of indole auxin metabolites in the excreta of IAA- and host plant-feeding *L. disponsi*: st-standard, exc-excreta, RFB-rape flower bud, RS-rape stem, CF-clover, flower, SV-sugar beet leaf vein.

value of each spot in the RFB-excreta was 0.02, 0.13, 0.18, 0.25 and 0.30. With the exception of the spot of Rf 0.02, they were in accord with those of IAA and the IAA metabolites in the excreta of the bugs fed with IAA-sucrose diet (IAA-excreta). When the colour strength was compared among the spots in a same test sample, however, the spot which was most prominent in colour was different between IAA- and RFB-excreta. In the former it appeared at Rf 0.18, but at 0.25 in the latter. In the case of solvent II, the Rf value of each spot in the RFB-excreta was 0.02, 0.06, 0.09 and 0.13, respectively, some of which corresponded with those of the IAA-metabolites in the IAA-excreta, though the prominent spot appeared at Rf 0.13 in the former but at Rf 0.02 and 0.06 in the latter. With solvent III, the Rf value of each spot in the RFB-excreta was 0.20, 0.32 and 0.76. These were equal to those of some of the IAA-metabolites in the IAA-excreta. Again the Rf value of the prominent spot

was different between the RFB- and IAA-excreta. From these results, it is clear that some indole compounds in the plant tissues pass through the gut in intact form and/or are metabolized in the gut to appear in the excreta.

Auxin activity of crude excreta of plant-feeding bugs

The significantly high auxin activity was detected in the crude excreta of rape flower bud-feeding, rape stem-feeding and clover flower-feeding bugs (Table 3), suggesting that some of the indole compounds are auxins and pass through the gut from plant tissues to excreta without losing some of the auxin activity.

Auxin activity of each spot on TLC-plate in RFB-excreta

Ten fractions of TLC were assayed for auxin activity. The crude excreta had again a significantly high auxin activity, whereas none of the fractions (no indole compound in the excreta) showed such a significantly high auxin activity, though the

Table 3. AUXIN ACTIVITY IN THE CRUDE EXCRETA OF PLANT-FEEDING *L. disponi*

	Excreta				
	Distilled water (DW) control	Rape flower bud	Rape stem	Clover flower	Sugar beet vein
Length of <i>Avena</i> coleoptile (mm)	10.86	11.19	11.13	11.05	10.88
<i>t</i> -test		S ^a	S ^b	S ^b	N.S.

^a Significant at 1% level to DW control.

^b Significant at 5% level to DW control.

Fig. 2. Auxin activity in TLC fractions of the excreta of IAA- and host plant-feeding *L. disponi*.

Avena coleoptiles in the solution including each indole compound tended to elongate more than those in water (control) (Fig. 2).

It was found that the bug ingested $15.3 \mu\text{l}$ of diet containing 0.1% IAA per day (HORI, 1979). As estimated according to this value, $80 \mu\text{l}$ of the excreta spotted on a TLC sheet corresponds to $55 \mu\text{l}$ of the original diet in the IAA equivalent. As shown in Fig. 2 and Table 5, the auxin activity of the IAA excreta decreased far from that of the IAA diet, indicating that the auxin activity of the IAA-metabolites is much lower than that of IAA itself. This may be the reason why a significant auxin activity was detected in the crude solution of the RFB-excreta (the total of the fractions) but not detected in each of the fractions.

Effect of IAA on mortality and feeding of bugs

The feeding of the bugs was inhibited significantly by 0.5% IAA but had a tendency to be stimulated by 0.1 and 0.05% IAA in the presence of sucrose. The mortality of the IAA-feeding bugs was not different from that of the control-bugs.

In the present study, it was found that the excreta of the bugs fed with natural host plants contained some indole compounds (indole auxins), Rf values of which corresponded with those of the IAA-metabolites in the excreta of the bugs fed with IAA. This fact suggests that IAA may be a main indole auxin which enters the gut of the bugs from the host plant tissues. But the pattern produced on a TLC sheet as expressed by the order of the colour strength among the spots was different between the IAA- and the RFB-excreta. This may be due to: (1) The metabolism of IAA derived from host plants in the gut is affected by some other plant substances which come into the gut with IAA, (2) Although IAA is ingested from host plants and metabolized in the gut in the same way as pure IAA, some other indole auxins (for example indole-3-acetyl-L-aspartic acid, glucobrassicin etc.) also come into the gut and are excreted without being metabolized, (3) Other indole auxins as well as IAA are metabolized in the gut and the mixture of their metabolites appears in the excreta. In any case, it is probable that indole auxins contained in host plants are ingested

Table 4. EFFECT OF IAA ON THE MORTALITY AND FEEDING OF *L. disponsi*

Diet	Mortality (%)	Quantity of feeding $\mu\text{l}/\text{bug}/\text{day}$	F-Test
Sucrose alone (control)	28.3	7.53	
Sucrose-IAA (0.5%)	20.0	5.40	S (5%)
Sucrose-IAA (0.1%)	23.3	8.38	N. S.
Sucrose-IAA (0.05%)	21.7	8.50	N. S.
Sucrose-IAA (0.1%)	23.3	7.32	N. S.

Table 5. COMPARISON OF AUXIN ACTIVITY BETWEEN IAA DIET AND THE EXCRETA OF IAA-FEEDING *L. disponsi*

	Distilled water (DW) control	IAA diet $80 \mu\text{l}$	IAA diet $55 \mu\text{l}$	IAA excreta $80 \mu\text{l}$
Length of <i>Avena</i> coleoptile (mm)	10.55	12.66 ^{a, b}	12.43 ^{a, b}	11.75 ^a

^a Significant at 1% level to DW control.

^b Significant at 5% level to IAA excreta.

by the bugs *L. disponi* and lower molecular indole compounds such as IAA, if present, combine with some substances (amino acids, sugars and so on) to be excreted. Moreover, higher molecular indole compounds such as indole-3-acetyl-L-aspartic acid and glucobrassicin, if present, should be excreted without being metabolized or by conjugating with some substances in the gut. This may be a detoxication mechanism of the bugs for indole auxins or a mechanism of the bugs preventing them from being absorbed into the haemolymph in natural condition.

ACKNOWLEDGEMENTS

The author is thankful to Prof. Y. NISHIJIMA of the Obihiro University for useful suggestions throughout this work. Thanks are also due to Dr. Y. MINO of the same University for valuable technical suggestions.

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