Accumulation of indole glucosinolates in *Psylliodes chrysocephala* L. -infested, or -damaged tissues of oilseed rape (*Brassica napus* L.)

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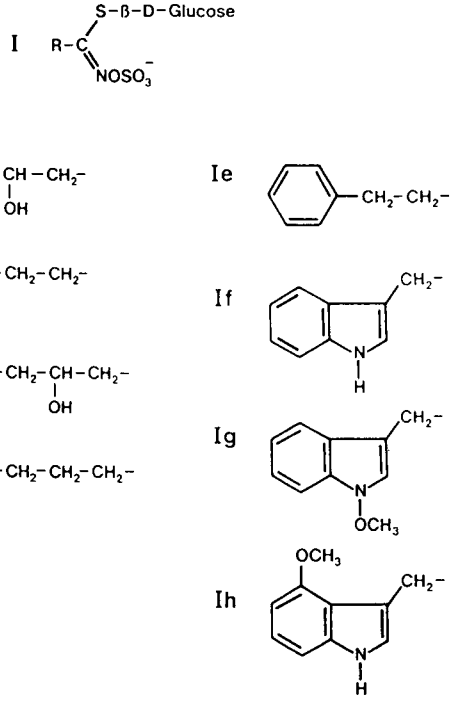
Summary. Infestation of oilseed rape (*Brassica napus* L.) by the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) leads to an overall reduction in the levels of aliphatic (alkenyl- and hydroxyalkenyl-) glucosinolates in the damaged tissue (lamina, petiole, stem) and a massive accumulation of indole glucosinolates. Whilst artificial damage (puncturing), with and without associated bacterial infection with an isolate from *P. chrysocephala,* led to such accumulation, this was less than that observed in the insect-infected situation.
Key words. Cabbage stem flea beetle; *Psylliodes chrysocephala* L.; oilseed rape; *Brassica napus* L.; glucosinolates.

Cruciferous plants have long been known to contain a family of secondary metabolites, the glucosinolates[1]. These compounds (fig., **I**) are hydrolized in the presence of an endogenous enzyme, thioglucoside glucohydrolase (EC 3.2.3.1), to produce glucose, sulphate and a variety of low molecular weight products possessing a diversity of chemical and biological properties[2,3]. Some of these plant products, notably isothiocyanates and oxazolidine-2-thiones, have been shown to exert protective characteristics against pathogens[1], as well as acting as stimuli for feeding and egg-deposition in insects[1,4–6].

Structures of glucosinolates found in oilseed rape petiole and lamina



I, general glucosinolate structure

Ia–Ih, structure of R group

The cabbage stem flea beetle (*Psylliodes chrysocephala* L.) attacks many brassicas, including oilseed rape. The adult female lays its eggs in the autumn in the soil near the plant and the larvae climb the plant stem and preferrentially infest the petioles. As part of a comprehensive study of the factors affecting, and the consequences of, such plant/insect interactions[7] we have investigated the effect of infestation by *P. chrysocephala* on the glucosinolate content of the petioles, and other tissues, of oilseed rape (*B. napus* L.).

Material and methods. Field- and laboratory-grown plants of oilseed rape (*Brassica napus* L. cv. Rafal) infested with larvae of the cabbage stem flea beetle (*P. chrysocephala* L.) were dissected after ten days and the petioles and laminae removed and freeze dried. Laboratory plants were grown in a controlled environment chamber at a photosynthetic photon flux density of 120 $\mu E\,m^{-2}\,s^{-1}$ and a light regime of 12 h light (16 °C), and 12 h dark (10 °C). The soil was a 3:1 peat-sand mixture containing 0.15 g KNO_3, 0.15 g K_2SO_4, 2.95 g Mg limestone, 1.20 g superphosphate, 2.35 g $CaCo_3$ and 0.37 g Fortone G (fritted trace elements containing B, Cu, Fe, Mn, Mo and Zn in unknown quantities) per liter of soil[8]. Plants were infested when their fourth leaf was emerging, watered every two days with tap water and given Hoagland's nutrient solution (25% strength) every 8 days.

Field plants were sown on 1 September, 1987 on a calcareous loam soil (pH 7.8) that had received 50 kg of each of N, K and P per hectare following a previous crop of winter wheat. Insect damage was seen in late October and plants harvested for analysis in mid-January, mid-February and mid-March, 1988.

Freeze-dried samples from 10 plants were combined, stored at − 40 °C, extracted and analysed in duplicate for individual glucosinolate content according to the method of Spinks et al.[9]; glucosinolate assignments were confirmed by HPLC/MS measurement[10] or by direct comparison with isolated standards. The detection limits were $< 0.01\ mg\,g^{-1}$ for glucosinolates, **Ia–Ic, If–Ih** and $< 0.02\ mg\,g^{-1}$, for **Id, Ie**.

Individual laboratory grown plants were also physically damaged by puncturing the petiole with a hypodermic syringe and infected with *Pseudomonas* spp. obtained from the surface of *P. chrysocephala*. Such infested and/or damaged tissue was collected after 10 days and treated as above.

Results and discussion. Table 1 shows typical results, which indicate that quantitative changes occurred following the infestation of rapeseed petioles (or stem and lamina – results not shown) with the beetle. In most cases such infestation led to a moderate reduction (10–30%) in total glucosinolate content, but occasionally (table 2) there are large increases (4.3 → 16.3 mg glucosinolate g^{-1} freeze dried tissue). However an examination of the levels of the individual glucosinolates, which may conveniently be grouped according to the presence of aliphatic, aromatic or indolic side chains (R, structure **I**), revealed that whilst there was an overall reduction in the aliphatic glucosinolates upon infestation, the levels of indole glucosinolates (and especially that of 3-indolylmethyl glucosinolate (**If**)) greatly increased. The same finding was observed for lamina and stem tissue.

The accumulation of secondary metabolites in response to microbial invasion, insect or mechanical damage is known to occur in many plant families, including the Leguminosae, Solanaceae and Compositae[11–13]. This is, however, the first report of the accumulation of glucosinolates in the shoots of members of the Cruciferae.

The difference in behaviour of the aliphatic, aromatic and indolic glucosinolates is consistent with the likely differing biosynthetic pathways for the latter compounds[1,3]. The accumulation of the indole glucosinolates is, perhaps, rather unexpected given the wide-ranging biological properties of the hydrolysis products of aliphatic and aromatic glucosinolates[1]. However, recent work indicates that the enzymatically-derived products of indole glucosinolate-breakdown possess a range of biological properties[14,15] and, as Butcher et al.[16] have shown, the indole glucosinolate content of the root tissue of swede (*B. napus* L.) increased (3–5-fold) following infection with the clubroot organism, *Plasmodiophora brassicae* Wor., but only 3-indolylmethyl glucosinolate (**If**) was affected.

In an attempt to determine the significance of infestation to indole glucosinolate accumulation, the effects of physical damage (puncturing) and wounding together with bacterial infection of oilseed rape petiole and lamina were compared with those produced by beetle infestation (table 2). Whilst the indole glucosinolates increased following insect infestation, other damage also lead to the accumulation of these compounds although the levels reached were less than that following infestation. It is also notable that the wounded petiole showed an elevated 2-hydroxybut-3-enyl glucosinolate (**Ia**) content.

Recently phytoalexins containing indole and 1-methylindole moieties have been identified in the Crucifer-

Table 1. Glucosinolate contents (mg g^{-1} freeze-dried material) of infested and non-infested petioles of field grown plants

	Glucosinolates* Aliphatic					Aromatic	Indolic				Total
	Ia	**Ib**	**Ic**	**Id**	Total aliph.	**Ie**	**If**	**Ig**	**Ih**	Total indoles	
−**	0.82	0.20	0.31	0.78	(2.11)	0.08	0.08	0.20	0.14	(0.42)	2.61
+	0.22	ND	0.12	0.02	(0.36)	ND	0.40	0.59	0.12	(1.11)	1.53
−	4.70	1.65	1.57	4.24	(12.16)	0.24	0.10	0.30	0.05	(0.45)	12.85
+	2.21	0.39	1.15	2.27	(6.02)	0.20	1.13	1.28	0.09	(2.50)	8.75
−	5.87	1.08	2.05	9.88	(18.88)	0.36	0.84	0.88	0.05	(1.77)	21.08
+	5.81	0.49	2.30	6.28	(14.88)	0.36	3.08	3.24	0.19	(6.51)	21.80

* For structure of these glucosinolates, see figure. ** +, sample infested with *P. chrysocephala*. −, sample not infested with *P. chrysocephala*. ND, not detected (see 'Material and methods' section for detection limits). **Ia,** 2-hydroxybut-3-enyl glucosinolate; **Ib,** but-3-enyl glucosinolate; **Ic,** 2-hydroxypent-4-enyl glucosinolate; **Id,** pent-4-enyl glucosinolate; **Ie,** 2-phenethyl glucosinolate; **If,** 3-indolylmethyl glucosinolate; **Ig,** 1-methoxy-3-indolylmethyl glucosinolate; **Ih,** 4-methoxy-3-indolylmethyl glucosinolate.

Table 2. Comparison of infestation, wounding and bacterial infection on glucosinolate content (mg g^{-1} freeze-dried material) of rape petiole and lamina, of laboratory grown plants

	Glucosinolates Aliphatic					Aromatic	Indolic				Total
	Ia	**Ib**	**Ic**	**Id**	Total aliphat.	**Ie**	**If**	**Ig**	**Ih**	Total indoles	
Non-infested petiole	0.85	0.91	0.35	1.45	(3.56)	0.42	0.23	0.08	0.05	(0.36)	4.43
Infested petiole	1.08	0.07	0.47	0.60	(2.22)	1.18	10.13	2.67	0.08	(12.88)	16.33
Wounded petiole	1.89	0.42	0.58	1.70	(4.59)	1.30	1.90	0.22	0.10	(2.22)	8.11
Wounded, infected* petiole	1.55	0.29	0.52	1.45	(3.81)	1.34	4.43	0.38	0.10	(4.91)	10.09
Non-infested lamina	0.08	0.13	0.20	0.72	(1.13)	ND	0.16	0.02	0.01	(0.19)	1.32
Infested lamina	0.10	ND	0.21	0.41	(0.72)	ND	1.00	0.07	0.07	(1.14)	1.86
Wounded lamina	0.09	ND	0.21	0.44	(0.74)	ND	0.73	0.01	0.04	(0.78)	1.52
Wounded, infected* lamina	0.14	ND	0.31	0.83	(1.28)	ND	0.92	0.01	0.04	(0.97)	2.25

* Petiole, lamina infected with *Pseudomonas* species isolated from *P. chrysocephala*. Glucosinolates, detection limits as for table 1.

ae[17–20], but these compounds do not appear to be hydrolysis products of indole glucosinolates **If** and **Ig**[17]. Studies are now in progress to examine whether indole glucosinolates and such phytoalexins may both be accumulated in response to cellular damage. The full significance of the findings reported here and their extrapolation to other bacterial infections and insect damage, has yet to be assessed. However the study of the physiological role of indole glucosinolates in plant/insect interactions may be facilitated by recent advances in methods for the analysis of individual glucosinolates[3] and by the availability of purified glucosinolate standards[21]. The above findings do, perhaps, call into question the reliability of analysing whole plant glucosinolates as a means of assessing or predicting insect/fungal resistance or susceptability. They also suggest that current ideas regarding the role and relative importance of indole glucosinolates in plants be re-evaluated.

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