ACCUMULATION OF GLUCOSINOLATES IN OILSEED RAPE LEAVES AS A RESULT OF FUNGAL INFECTION

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INTRODUCTION

Crucifer species, including winter oilseed rape (*Brassica napus* L. ssp. *oleifera*), contain glucosinolates, which are precursors for a range of compounds implicated both in resistance to disease and in influencing the behaviour of oilseed rape pests. Various authors (Lammerink et al. 1984; Koritsas et al. 1989; Birch et al. 1990) have shown that glucosinolates accumulate in oilseed rape tissues following infestation by pests or simulated pest-damage. In this study, we show that similar accumulation occurs during infection by the fungal pathogen, *Alternaria brassicae* (Berk.) Sacc., and that the pattern of accumulation differs between cultivars and between leaves of different ages.

MATERIALS AND METHODS

Plants of oilseed rape cultivars Bienvenu and Cobra were raised in a controlled environment room at 18/16°C day/night temperature, 80-90% relative humidity, twelve hour daylength. Plants at growth stage 1.08 - 1.09 (Sylvester-Bradley 1985) were sprayed with a suspension of mycelium of *A.brassicae* in malt extract broth (Oxoid) and maintained at high humidity; control groups were sprayed with malt extract broth alone. Fourth and sixth leaves were removed from groups of five plants at the time of inoculation and at intervals over the following twenty days; disease severity was estimated using image analysis, then leaves were analysed for glucosinolate concentration by HPLC. Further details of experimental techniques are given elsewhere (Doughty et al. 1991).

RESULTS

Glucosinolate concentrations were similar in corresponding leaves of Bienvenu and Cobra at the time of inoculation, and they declined in uninoculated leaves over the course of the experiment (data not shown). However, in inoculated material they generally increased, especially in sixth leaves. Tables 1 and 2 show the glucosinolate concentration in fourth and sixth leaves, respectively, of the two cultivars at five and sixteen days after inoculation. Among the glucosinolates, aliphatics accumulated more rapidly than other types in sixth leaves of cv. Bienvenu, then declined, but they remained at low concentrations in sixth leaves of cv. Cobra and in fourth leaves of both cultivars. Indolyl glucosinolates accumulated more slowly, but only in fourth and sixth leaves of cv. Bienvenu. Aromatic glucosinolates reached maximum concentrations about 16 days after inoculation in fourth and sixth leaves of both cultivars. Within these groups, individual glucosinolates accumulated to different degrees; for example, among indolyl compounds, 3-indolylmethyl-and 1-methoxy-3-indolylmethyl glucosinolates increased markedly in inoculated sixth leaves but 4-hydroxy-3-indolylmethyl glucosinolate did not. Of the aromatics, only 2-phenylethyl glucosinolate accumulated in response to infection.

Disease symptoms were initially greater on Cobra, but as infection progressed, symptom severity was comparable on the two cultivars. However,

Table 1 Glucosinolate concentration (μmol/ml) in tissue water of inoculated (+) and uninoculated (-) fourth leaves, cultivars Bienvenu and Cobra, at 5 and 16 days after inoculation.

	5 days				16 days			
	Bienvenu		Cobra		Bienvenu		Cobra	
	+	-	+	-	+	-	+	-
Aliphatic								
3-butenyl	0.03	0.02	0.04	0.05	0.05	0.08	0.02	0.01
4-pentenyl	0.02	0.02	T	0.01	0.01	0.03	0.01	T
2-hydroxy-3-butenyl	0.05	0.03	0.02	0.03	0.04	0.11	0.02	0.01
Total	0.10	0.07	0.06	0.09	0.10	0.22	0.05	0.02
Aromatic								
2-phenylethyl	0.17	0.05	0.81	0.04	1.57	0.22	0.57	0.03
p-hydroxybenzyl	T	T	T	T	T	0.01	T	T
Total	0.17	0.05	0.81	0.04	1.57	0.23	0.57	0.03
Indolyl								
3-indolylmethyl	0.04	0.01	0.08	0.01	0.22	0.05	0.07	0.01
4-hydroxy-3-indolylmethyl	T	T	T	T	0.01	0.01	T	T
1-methoxy-3-indolylmethyl	0.01	T	0.01	T	0.10	T	0.01	T
Total	0.05	0.01	0.09	0.01	0.33	0.06	0.08	0.01
Total Glucosinolates	0.32	0.13	0.96	0.14	2.00	0.51	0.70	0.06

T = trace or undetectable

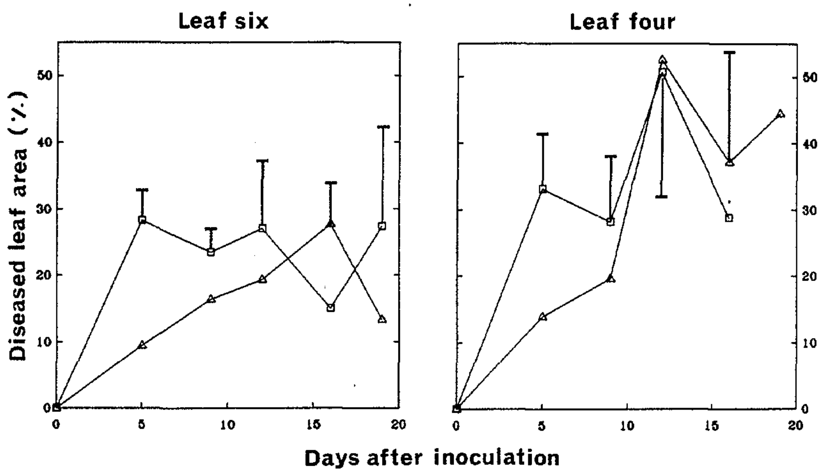
Table 2 Glucosinolate concentration (μmol/ml) in tissue water of inoculated (+) and uninoculated (-) sixth leaves, cultivars Bienvenu and Cobra, at 5 and 16 days after inoculation

	5 days				16 days			
	Bienvenu		Cobra		Bienvenu		Cobra	
	+	-	+	-	+	-	+	-
Aliphatic								
3-butenyl	0.52	0.27	0.21	0.41	0.20	0.23	0.22	0.17
4-pentenyl	1.15	0.49	0.13	0.41	0.21	0.30	0.10	0.07
2-hydroxy-3-butenyl	0.77	0.26	0.11	0.32	0.26	0.29	0.12	0.08
Total	2.44	1.02	0.45	1.14	0.67	0.82	0.44	0.32
Aromatic								
2-phenylethyl	0.43	0.19	0.80	0.15	1.99	0.10	0.87	0.11
p-hydroxybenzyl	0.09	0.02	T	0.01	0.06	0.01	T	0.01
Total	0.52	0.21	0.80	0.16	2.05	0.11	0.87	0.12
Indolyl								
3-indolylmethyl	0.07	0.05	0.15	0.13	0.31	0.02	0.16	0.03
4-hydroxy-3-indolylmethyl	T	T	T	T	T	T	T	T
1-methoxy-3-indolylmethyl	0.01	T	0.02	T	0.24	T	0.02	T
Total	0.08	0.05	0.17	0.13	0.55	0.02	0.18	0.03
Total Glucosinolates	3.04	1.28	1.42	1.43	3.27	0.95	1.49	0.47

T = trace or undetectable

the proportion of leaf area affected was consistently less on sixth than on fourth leaves, reaching a maximum of about 50% on fourth leaves, but less than 30% on sixth leaves (Figure 1).

Fig. 1 Development of disease on sixth and fourth leaves of cultivars Bienvenu (△—△) and Cobra (□—□) after inoculation with *Alternaria brassicae*. Bar lines represent the standard error of the mean difference.



DISCUSSION

Our study has demonstrated that glucosinolates accumulate in leaves of oilseed rape in response to infection by *A.brassicae*, and that the nature and magnitude of this response differs between cultivars. Moreover changes in glucosinolate concentration in the cell water of inoculated leaves may have been increasingly underestimated as developing lesions decreased the number of metabolically active cells.

After an initial rapid rise, the glucosinolate content of an individual, uninoculated developing leaf declines steadily until it reaches a basal level (Porter et al. 1991). It appears from these data that as leaves age, they also lose the ability to accumulate glucosinolates, and especially aliphatics, in response to infection.

Despite differences between cultivars in the pattern and extent of glucosinolate accumulation, and in contrast with the apparently greater susceptibility of Cobra in the field (Rawlinson et al. 1989), inoculation with *A.brassicae* caused similar symptoms on the two cultivars under the conditions of the present study. Sixth leaves, which accumulated more glucosinolate, were less diseased than fourth leaves, but there are many features of younger leaves which are thought to confer resistance to *A.brassicae* (e.g. Skoropad and Tewari 1977). Use of a concentrated inoculum meant that symptoms extended to up to 50% of the area of fourth leaves, despite the accumulation of certain types of glucosinolates. Studies are in progress to investigate if enhanced glucosinolate concentrations hinder infection at lower inoculum levels.

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