RATE OF RELEASE OF ALLYL ISOTHIOCYANATE BY INTACT AND DAMAGED ORIENTAL MUSTARD PLANTS AND IMPLICATIONS FOR HOST PLANT LOCATION BY INSECTS

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INTRODUCTION

All plants of the Brassicaceae contain one or more glucosinolates (GS). Cells of these plants contain myrosinase which facilitates hydrolysis of GS to isothiocyanates (IC) or mustard oils, thiocyanates and nitriles following tissue damage. As IC have toxic effects on mammals, insects and microorganisms, their formation in response to damage may serve as a defense mechanism. However, a number of insect species feed primarily or exclusively on plants containing GS. Some crucifer-specialist insects are stimulated to feed or oviposit in the presence of GS. Some are attracted by one or more of the IC (Chew, 1988).

GS concentrations and distribution in different plant parts and phenological stages have been determined for several crucifers by direct quantification (Sang et al., 1984; McGregor and Love, 1987; McGregor, 1988) or by quantification of hydrolysis products from macerated plant tissue (Cole, 1980). However, almost no information is available on IC release rates from living plants. In this paper, we measure release rates of allyl IC from Oriental mustard, Brassica juncea (L.) Czerniak.

MATERIALS AND METHODS

Certified seeds of Oriental mustard cv. Domo (except where indicated as cv. Cutlass) were planted in cylindrical glass vessels (15 cm I.D. X 30 cm in height) using a soil-less mix, 10 pellets of a slow-release fertilizer (12-12-12), and watered as needed in a growth cabinet with a 16:8 L:D photoperiod at 21 ± 1°C, 65 ± 5% RH. The same growing conditions were maintained during volatile collections. Volatile collections were carried out three times (referred to as Collections I-III), starting with plantings of 7, 6 and 5 vessels, respectively.

For volatile collections the glass vessels were sealed with an acrylic top fitted with a rubber gasket and were connected to a closed loop stripper (Model CLS-1, Tekmar Co., Cincinnati, Ohio) by 3-mm (I.D.) polyethylene tubing. Headspace vapours were circulated through a glass cartridge containing 5 mg activated charcoal maintained at 50°C to prevent water condensation.

Volatiles were collected using a closed (Collection I, II) or open loop system (Collection III). The latter method, accomplished by disconnecting the inlet air tube from the apparatus and connecting to a fresh filtered air source, was

carried out to ensure that changing O_2 and CO_2 levels did not affect allyl IC release. Plants were cut at the soil surface, counted and weighed after the final volatile collection.

Carbon filters were eluted with four 20-ml aliquots of dichloromethane. To clean between samples, filters were rinsed with dichloromethane and flushed with heated nitrogen gas for 24 h. Dichloromethane was also circulated for 2 h through the stripping apparatus. Samples were analyzed by GC/MS (Finnigan 4000) using internal (10 ng/µl chlorobenzene) and reference standards. Blanks (soil only) were also analyzed and mean values per collection (n=3) were subtracted from values for other samples. Some samples were lost or severely depleted as a result of leaky storage vial lids and these were excluded from the analyses.

Volatiles were collected from each vessel of plants at 5 d after seeding (seedling), 14 d (rosette), and 28 d (bud stage). There were 60 plants/vessel at 5 d, which were thinned to nine plants after the first volatile collection, and in collection I only, to four plants following the 14-d collection. After the 28-d collection, plants were damaged on each of the following two days, and after each damage, volatiles were collected separately for the first hour following damage and the next 23 h. In Collection I, leaves were damaged by removing the distal half with scissors. In Collections II and III, a single 8-mm diameter hole/leaf was made with a paper hole punch. On the first day, one leaf/plant was damaged and on the second day an additional two were damaged, of approximately six leaves in total/plant.

RESULTS

The rate of allyl IC release per plant increased by an order of magnitude from 5 to 28 d (Table 1). The increase resulted from increased biomass as there was an actual two- to ten-fold decrease in release rates on a gram wet weight basis with increasing age (Table 1b). Mean plant wet weights in Collections I-III varied from .05-.10 g at 5 d, .25-1.33 g at 14 d and 2.6-8.0 g at 28 d.

Cutlass, the second variety tested at 5 d after seeding, did not differ in any consistent manner from Domo (Table 1). Both varieties displayed considerable variability in release rates both within and between collections. In fact, this was true of all plant phenological stages with or without damage. Variation in release rates could not be attributed to the differences in collection methodologies as there were no consistent variations between collections.

The release rate increases following artificial damage were dramatic (Tables 1). There appeared to be a higher rate of allyl IC release with the leaf-cutting method (Collection I) than with the hole-punch method (Collections II, III), but only for the first hour of the initial damage. The high rate of release of allyl IC following damage ceased in less than 1 h. In the following 23 h, the rates were similar to pre-damage levels. After the additional damage, release rates again climbed for the first hour, but much less than on the previous day. They were only 3 (Collection I) to 32% (Collection II) of the rates in the initial damage even though the additional damage was approximately twice as

extensive. Again, pre-damage rates were attained over the next 23 h. These were consistently lower than the 23 h period of the previous day.

DISCUSSION

To our knowledge, no quantitative data is available on production of allyl IC or other IC in intact crucifers. Allyl IC is a hydrolysis product of allyl GS, the predominant GS in B. juncea (Cole, 1976). The levels of production measured here for intact B. juncea vary greatly between replicates, with mean levels which increase an order of magnitude from 5 d to 28 d. Wallbank and Wheatley (1976) noted variation over three orders of magnitude in allyl IC content of macerated roots of mature cauliflower. McGregor and Love (1987) measured levels of allyl GS in B. juncea cv. Domo. From 5-28 d after seeding, levels of allyl GS measured were proportional to our measurements of allyl IC: an increase over one order of magnitude, with a slight decline on a gram-weight basis over the same period.

Allyl IC release can be calculated as a percentage of total allyl GS present in the plant. Approximate mean allyl IC release rates from our data are 1, 2, 15 and 2000 ng/day per plant for 5, 14, 28 d, and the first hour following initial damage respectively. Allyl GS content of B. juncea cv. Domo is approximately .2, 2 and 20 µmoles per plant at 5, 14 and 28 d, respectively (McGregor and Love, 1987). Taken together, this suggests that .005, .001, .0008, and .1% per day per plant of allyl GS is released in the form of allyl IC at 5, 14, and 28 d, and for the first hour following initial damage, respectively. The levels for intact plants are two-three orders of magnitude lower than the 0.7% or 7 µg per day estimated by Finch (1978) for total aglucones released by individual intact 10-week-old rape plants, B. napus L., although data are not presented in his paper.

Previous studies indicate that decline of GS levels in crucifer seedlings and subsequent increase in other tissues are separate events, usually involving different types of GS in different tissues (Cole, 1980; Sang et al., 1984; McGregor, 1988). Release of allyl IC from intact plants probably reflects metabolic turnover of GS.

We observed massive increases in allyl IC production following damage. Large releases of IC following leaf-cutting (Wallbank and Wheatley, 1976) or maceration (Cole, 1976; Cole, 1980, Tollsten and Bergström, 1988) have previously been demonstrated in several crucifers including B. juncea. However, not all crucifers release large amount of IC upon damage (Cole, 1976). It is particularly interesting that allyl IC release can be enormous even after minor plant damage, that allyl IC release returns to close to pre-damage levels within less than one hour after damage, and that the plant does not recover its ability to release allyl IC within 24 h, as demonstrated by the small release rates after additional damage. The damage done to the plants was not severe, particularly in Collections II and III. Damage to plants by aphids and flea beetles may cause increases in total or indole GS, respectively (Lammerink et al., 1984; Koritsas et al., 1989), indicating that damage may affect GS

metabolism. The lower rates of allyl IC release on the second day of damage in our experiments appear to support these findings, as they indicate that GS concentration or hydrolysis is somehow altered by previous plant damage.

Quantification of release rates to which insects respond are scarce. The cabbage root fly is known to respond in the field to baits releasing a few grams per day of allyl IC, and to baits in the wind tunnel of 32-132 mg/day (Wallbank and Wheatley, 1979). Minimum thresholds have now been established in field trapping experiments for the northern false chinch bug of 0.4 mg/day for three IC including allyl IC, 0.04 mg/day for another IC (ethyl 4-isothiocyanatobutyrate) and no response to still another IC (2-phenylethyl isothiocyanate; Pivnick et al., in press). In flea beetles, minimum thresholds are approximately 0.4 mg/day of allyl IC for field trapping, and beetles respond to baits from less than 2 m away (Pivnick et al., unpublished).

It is still not clear how IC are used by insects in host plant location, or whether indeed host plant location is the main function of insect response to IC. Based on the threshold levels cited above, individual plants would not be attractive even immediately following damage of the kind we carried out, although fields of cruciferous crops would be attractive. Assuming release of approximately 2 µg/day following initial damage, it would require a group of 200 mustard plants to attract nearby flea beetles or false chinch bugs. For intact bud-stage plants, it would require 27,000 plants assuming an average release of 15 ng/day of allyl IC and that no damage normally occurs in the field. These estimates will differ for different insect species and kinds of IC, as described above. Since we have measured release following only two different types of damage, other types of damage may provide different patterns of release. Nor do we know the chronology of release rates within the first hour after damage. Also, response to IC in field trapping is a function of insect density, wind conditions, distance from traps and insect search behaviour. Flea beetles rarely fly to baited traps in the Canadian prairies because of high winds on most days (Pivnick, personal observation). The cabbage root fly will orient to a plant of cabbage from up to 10 m downwind (Hawkes, 1974). It is likely that lower release rates will be attractive when insects are very close to the source, or when flying on calm days.

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Table 1. The daily rate of release of allyl isothiocyanate from potted Brassica juncea on (a) an individual plant basis and (b) a gram wet weight basis.

Time from seeding and plant condition[1]	Release of allyl IC (ng/day)[2] Collection I	Collection II	Collection III
(a)			
5 d	2.6 ± 1.0 (7)	0.4 ± 0.2 (6)	------
5 d-Cutlass	0.4 ± 0.3 (7)	1.7 ± 1.4 (6)	------
14 d	3.3 ± 1.7 (7)	0.5 ± 0.4 (5)	1.3 ± 0.9 (4)
28 d	------	20.7 ± 7.0 (5)	8.8 ± 4.5 (5)
Initial damage - 1 h	5750 ± 4110 (5)	649 ± 216 (5)	------
- next 23 h	17.9 ± 11.6 (5)	21.0 ± 7.2 (5)	20.8 ± 18.8 (2)
Additional damage - 1 h	172 ± 88 (5)	207 ± 56 (5)	129 ± 60 (2)
- next 23 h	1.9 ± 1.2 (5)	12.1 ± 5.9 (5)	6.4 ± 5.2 (3)
(b)			
5 d	36.3 ± 14.3 (7)	5.4 ± 2.0 (6)	------
5 d- Cutlass	5.0 ± 2.8 (7)	24.6 ± 20.8 (6)	------
14 d	7.2 ± 4.3 (7)	0.7 ± 0.6 (5)	3.1 ± 2.2 (4)
28 d	------	3.5 ± 1.3 (5)	2.1 ± 1.0 (5)
Initial damage - 1 h	1050 ± 530 (5)	104 ± 32 (5)	------
- next 23 h	3.2 ± 1.7 (5)	3.5 ± 1.2 (5)	4.2 ± 3.9 (2)
Additional damage - 1 h	54.3 ± 28.9 (5)	34.9 ± 8.6 (5)	31.4 ± 7.6 (2)
- next 23 h	0.7 ± 0.5 (5)	1.8 ± 0.8 (5)	1.4 ± 1.0 (3)

[1] The cultivar Domo was used except where otherwise indicated.
[2] $\bar{X}$ ± SE; sample sizes are in parentheses.