9. Asen, S., Stewart, R. N. and Norris, K. H. (1979) *Phytochemistry* **18,** 1251.
10. Anderson, D. W., Gueffroy, D. E., Webb, A. D. and Kepner, R. E. (1970) *Phytochemistry* **9,** 1579.
11. Krause, J. and Strack, D. (1979) *Z. Pflanzenphysiol.* **95,** 183.
12. Bloom, M. and Geissman, T. A. (1973) *Phytochemistry* **12,** 2005.
13. Yoshitama, K. and Hayashi, K. (1974) *Bot. Mag. Tokyo* **87,** 33.

Phytochemistry, 1981, Vol. 20, pp. 187–188. Pergamon Press Ltd. Printed in England.

0031–9422/81/0101–0187 $02.00/0

PHYTOALEXIN ACCUMULATION IN CHLOROFORM-TREATED COTYLEDONS OF *PHASEOLUS VULGARIS*

JOHN A. BAILEY and MONIQUE BERTHIER*
Long Ashton Research Station, University of Bristol, Bristol, BS18 9AF, U.K.

(*Received* 28 *February* 1980)

Key Word Index—*Phaseolus vulgaris*; Leguminosae; isoflavonoids; phytoalexins; chloroform; elicitor.

Phytoalexins are antifungal compounds produced by plants in response to infection by micro-organisms [1, 2] and viruses [3, 4], and after treatment with various chemicals [5, 6]. Recent studies with bean, *Phaseolus vulgaris*, have suggested that when tissue is partially damaged, e.g. by infection or partial freezing [7] or by treatment with $HgCl_2$ [8], a metabolite (constitutive elicitor) is released from the damaged cells which instigates isoflavonoid phytoalexin biosynthesis in the adjacent living tissue. As part of an investigation into the relationship between death of plant cells and subsequent accumulation of isoflavonoids, we have assessed the effects of $CHCl_3$ vapour, which kills cells very rapidly, on cotyledons of *P. vulgaris*.

After exposure to $CHCl_3$ for 1–5 min, cotyledons showed a gradual browning of their surfaces after incubation for between 10 and 72 hr. Isoflavonoids accumulated in the discoloured cotyledons and the amounts of phytoalexin produced became greater as the damage progressed (Table 1). Phaseollin, phaseollinisoflavan and kievitone were most abundant, being present at concentrations up to 96 μg/g cotyledon. Similar quantities of phaseollin were produced by cotyledons treated with $HgCl_2$ [8]. Phaseollidin and 2 hydroxygenistein were also detected (UV and MS [9]); however, their concentrations were always less than 3 μg/g cotyledon. Cotyledons treated with $CHCl_3$ for more than 10 min became totally flaccid and did not become pigmented or produce any of the above compounds. No isoflavonoids were detected in undamaged cotyledons.

Chloroform is very volatile and is unlikely to persist within the treated tissues. The results therefore support the suggestion, made earlier, following studies with $HgCl_2$ [8], that the accumulation of phytoalexins can be a direct consequence of the death of superficial cells of bean cotyledons.

* Present address: Département de Biologie Végétale, Université Claude Bernard, 43 Boulevard du 11 Novembre 1918, F-69-Villeurbanne, Lyon, France.

Table 1. The effect of chloroform on the accumulation of isoflavonoids in cotyledons of *Phaseolus vulgaris*

Period of treatment with $CHCl_3$ (min)	Period of incubation at 25° (hr)	Concentration of isoflavonoid, μg/g		
		Phaseollin	Phaseollin-isoflavan	Kievitone
3	0	–	–	+
3	24	+	+	10
3	48	12	15	32
3	72	29	65	30
3	96	20	54	44
3	144	26	96	28
0	72	–	–	–
1	72	–	10	6
2	72	9	30	8
4	72	30	60	46
5	72	44	38	71

Four-day-old cotyledons were treated with $CHCl_3$ and incubated at 25°. Concentrations of isoflavonoids were measured by UV spectrophotometry.

– indicates compound not detected by spraying chromatograms with diazo-*p*-nitroaniline; + indicates compounds were detected with diazo-*p*-nitroaniline but there was insufficient for UV spectrophotometry, i.e. less than 3 μg/g tissue.

EXPERIMENTAL

Seeds of *Phaseolus vulgaris* cv Kievitsboon Koekoek were surface-sterilized in 10% w/v NaClO for 20 min, washed in H_2O and placed between moist paper for 4 days at 25°. The testas were removed and individual undamaged cotyledons were placed on a glass plate. This was then supported 5 cm above 200 ml $CHCl_3$ contained in a beaker (2 l.), which had been covered with glass and allowed to equilibrate at room temp. for 1 hr. Samples of cotyledons (4–5 g) were removed after periods up to 30 min and incubated at 25° for up to 144 hr. The cotyledons were ground in a mortar, extracted in EtOH (95%) and after purification by TLC the concns of isoflavonoids were measured by UV [10].

Acknowledgement—M.B. wishes to thank the British Council for financial support.

REFERENCES

1. Deverall, B. J. (1977) *Cambridge Monogr. Exp. Biol.* No. 19.
2. Harborne, J. B. and Ingham, J. L. (1978) in *Biochemical Aspects of Plant and Animal Coevolution* (Harborne, J. B., ed.) pp. 343–405. Academic Press, London.
3. Bailey, J. A. and Ingham, J. L. (1971) *Physiol. Plant Pathol.* **1**, 451.
4. Klarman, W. L. and Hammerschlag, F. (1972) *Phytopathology* **62**, 719.
5. Cruickshank, I. A. M. (1963) *Annu. Rev. Phytopathol.* **1**, 351.
6. Yoshikawa, M. (1978) *Nature* **275**, 546.
7. Hargreaves, J. A. and Bailey, J. A. (1978) *Physiol. Plant Pathol.* **13**, 89.
8. Hargreaves, J. A. (1979) *Physiol. Plant Pathol.* **15**, 279.
9. Biggs, R. (1975) *Aust. J. Chem.* **28**, 1389.
10. Bailey, J. A. (1974) *Physiol. Plant Pathol.* **4**, 477.