Bot. Bull. Academia Sinica (1989) **30:** 107-115

Phytoalexin accumulation in plant tissues of *Brassica* spp. in response to abiotic elicitors and infection with *Leptosphaeria maculans*

J.S. Dahiya[1] and S.R. Rimmer[2]

[1]*ABI-Biotechnology Incorporated, 40 Scurfield Blvd., Winnipeg, Manitoba, R3Y 1G4*

[2]*Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2*

(*Received September 14, 1988; Accepted January 6, 1989*)

Abstract. Accumulation of the phytoalexins, methoxybrassinin and cyclobrassinin, in *Brassica* plant tissues was studied following induction by abiotic elicitors and compared with accumulation after inoculation with pycnidiospore suspensions of both aggressive and non-aggressive isolates of *Leptosphaeria maculans*, the causal fungus of blackleg/stem canker disease of cruciferous plants, The most effective elicitor, silver nitrate, induced considerably more phytoalexins than did fungal infection. Both methoxybrassinin and cyclobrassinin were found to accumulate in *Brassica* tissues in response to infection with a non-aggressive isolate of *L. maculans*, whereas only cyclobrassinin was recorded in tissues challenged with an aggressive isolate. Resistant germ lines accumulated more phytoalexins than the susceptible ones. Furthermore, the quantity of both phytoalexins depended on plant age, incubation temperature and method of elicitor application.

Key Words: *Brassica* spp.; Cyclobrassinin, *Leptosphaeria maculans*; Methoxybrassinin; ^{1}H-NMR Nuclear Overhauser Enhancement (NOE) and mass spectral analysis; Phytoalexins; UV.

Introduction

Phytoalexins are low molecular weight antibiotics that are both synthesized by, and accumulate in plants upon exposure to micro-organisms (Paxton, 1981). These antimicrobial substances are known to be produced in leaves, stems, cotyledons and hypocotyls (Ingham, 1982), in response to wounding (Rahe and Arnold, 1975), to interactions with micro-organisms or to treatment with certain chemical substances (Perrin and Cruickshank, 1965). The inducing substances are classified into abiotic and biotic elicitors. Abiotic elicitors are substances or conditions which do not occur in living tissues while biotic elicitors are substances derived from the pathogen or which are present in the plant tissue itself (Darvill and Albersheim, 1984). Phytoalexin accumulation is associated with disease resistance based on the observation that rapid accumulation of large amounts of phytoalexins occurs in resistant plants whereas in susceptible plants there is either delayed or smaller phytoalexin accumulation in response to pathogenic infections.

In addition to micro-organisms, a wide range of unrelated chemical agents also cause the accumulation of phytoalexins (Hadwiger and Schwochau, 1971; Hargreaves, 1979; Yoshikawa, 1978). It is difficult to envisage how such a diverse array of compounds can stimulate phytoalexin synthesis directly. A more plausible explanation, proposed by Van Etten and Pueppke (1976), is that abiotic agents cause the accumulation of phytoalexins through their ability to injure or kill plant cells and that it is this disruption of cellular metabolism that leads in some way to the production of

phytoalexins. Thus it is possible that micro-organisms and abiotic agents cause phytoalexin accumulation by a similar mechanism. Accumulation of methoxybrassinin and cyclobrassinin as phytoalexins in oilseed rape tissues (*Brassica napus*) and other cruciferous species. following infection with *Pseudomonas cichorii* and *Alternaria brassicae* has been reported earlier (Takasugi *et al.*, 1986; Conn *et al.*, 1988)

The aim of this present study was to investigate the ability of abiotic elicitors and both aggressive and non-aggressive isolates of *Leptosphaeria maculans*, the causal fungus of blackleg disease of crucifers, to induce phytoalexin production in oilseed *Brassica* cultivars.

Materials and Methods

Plant Material and Fungal Cultures

Seeds of cultivars of *Brassica napus* L., *Brassica rapa* L. and *Brassica juncea* (L.) Czern & Coss. were obtained from the departmental germ line collection of *Brassica* spp. Fungal isolates of *Leptosphaeria maculans* (Desm.) Ces & de Not. (anamorph=*Phoma lingam* (Tode ex Fr.) Desm.) used in the present studies were from a blackleg nursery maintained near Elgin, Manitoba, Canada. Plants were grown in soil mix peat at 25°C in a growth cabinet with 16 hours daylight. Fungus (*L. maculans*) was cultivated on V8 agar media at 25°C for 12 days.

Induction and Isolation of Phytoalexins

Detached leaves or stem segments were surface disinfected with 0.1% (w/v) mercuric chloride solution, rinsed (5x) with sterile distilled water. Longitudinal sections of the stem segments (1 cm in length) were made aseptically with a razor blade before transferring them onto moist sterile filter paper contained in a sterile petri plate. Fungal inoculum (50 μl) consisting of 10^6 pycnidiospores ml^{-1} in sterile distilled water was applied to the adaxial leaf or cut stem surfaces with a hypodermic syringe. Superficial wounds were made on the leaf surface before applying the inoculum to facilitate penetration of the leaf tissue by the fungus. The plates were incubated for 12 days at 25°C in the dark, diffusate from the leaf inoculations was collected (at the end of incubation), concentrated *in vacuo*, and filtered prior to chromatographic analysis. Leaf tissue underlying inoculum or the upper 1 mm of stem tissue was extracted by vacuum infiltration in methanol. Extracts were diluted with an equal volume of water and partitioned against diethyl ether. Compounds were purified by preparative HPLC.

HPLC Analysis

Samples (1 ml) were injected into an HPLC instrument (Beckman Model 100A) consisting of an Altex pump and injection valve, a preparative column (25× 1.0 cm, i.d.) of Ultramax 5C 18 (Terrochem Ltd., Edmonton, Alberta, Canada), an LC-UV detector set at λ267 nm and a Hewlett Packard hertz recorder. The solvent used was a mixture of acetonitrile and deionised water (70:30, v/v), with a flow rate of 3.0 ml min^{-1}. Active fractions, recognized initially by bioassay on silica gel TLC (Terrochem Ltd., Edmonton, Alberta, Canada) with *Cladosporium cucumerinum* (Bailey and Burden, 1973), and subsequently by retention time [Fig. 1] and absorption of light at 267 nm, were collected and dried. The fractions 1 and 2 were crystallized from aqueous methanol. Quantitative analysis of the phytoalexins in the crude mixtures was done by analytical HPLC using the column (Ultramax 5 C-18, 25×0.46 i.d.) with a flow rate of 1.5 ml min^{-1}, and isoprenylated genistein as the internal standard.

Identification and characterization of the purified compounds was done through UV, NMR, and mass spectral analysis.

Spectral Analysis

UV spectra were recorded in absolute methanol with a Varian XL-200 spectrophotometer. Mass spectra were measured with a VG analytical 11-250J by direct probe insertion. ^{1}H-NMR (400 MHz) spectra were recorded on a Bruker WH-90 with TMS as an internal standard.

For comparative studies authentic samples of methoxybrassinin and cyclobrassinin were supplied by Dr. M. Takasugi, Department of Chemistry, Hokkaido University, Sapporo, Japan.

Abiotic Elicitation of Phytoalexins

For abiotic elicitation of phytoalexins different heavy metal salts, silver nitrate (10^{-3}M), cupric chloride (10^{-2}M), lead nitrate (10^{-3}M), zine sulphate (10^{-2} M), nickel nitrate (10^{-3}M) and mercuric chloride (10^{-3}M) were used. The procedure was the same as

described earlier for the fungus, except that in the latter case the chemical solution (50 μl) was used in place of the fungal spore preparation.

UV irradiation was done for 20 min under an UVIS apparatus (Chromato-Vue Model C-3), Ultraviolet Products Inc., San Gabriel, California, USA). The lamps (254 nm) were set 15 cm from the leaves or stem pieces. After treatment, leaves or stem tissues were incubated at 25°C in humidified petri dishes in darkness for the period mentioned earlier. Extraction and estimation of phytoalexins was done as described earlier.

Results

Identification of Phytoalexins

Compound 1 with HPLC retention time 5.6 min [Fig. 1] was obtained as yellow oil. The mass spectral analysis gave m/z (rel. int.) 266 (10), ($C_{12}H_{14}N_2OS_2$ requires 266.0210 found 266.0231) and other fragment ions 235 (68, M-OMe), 218 (26, M-CH_4S), 160 (100), 145 (18), 129 (60) and 91 (23, $C_2H_3S_2$). Methanolic solution of the purified compound had absorbance maxima λ_{max}^{MeOH}: nm (log ϵ) at 218 (4.39), 267 (4.21) and shoulders at 287 (3.78) and 297 (3.46). ^{1}H-NMR spectral analysis (400 MHz $CDCl_3$) in deuterated chloroform assigned by spin decoupling and NOE (nuclear overhauser enhancement) experiments indicated five aromatic protons at δ 7.17 (ddd, J=8.0, 8.0 and 2.0 Hz, 5-H), 7.36 (ddd, J=8.0, 8.0 and 2.0 Hz, 6-H), 7.38 (5, 2-H) 7.48 (dd, J=8, 2.0 Hz, 7-H) and 7.66 (dd, J=7.8, 2.0 Hz, 4-H). Singlets at δ 4.10 indicated the presence of a methoxyl group (-OMe). Placement of the side chain at C-3 was supported by NOE of 2- and 7-H on irradiation at δ 4.10 (1-OMe). Presence of methylene (-CH_2-) in the side chain was confirmed by a doublet at δ 5.06 (d, J=4.0 Hz). One amide (>NH) group in the side chain was indicated by a broad singlet at δ 7.01 and a singlet at δ 2.67 indicated the presence of a thiomethyl (-SMe). Since the methylene proton signal changed to a singlet on D_2O exchange, the meth-

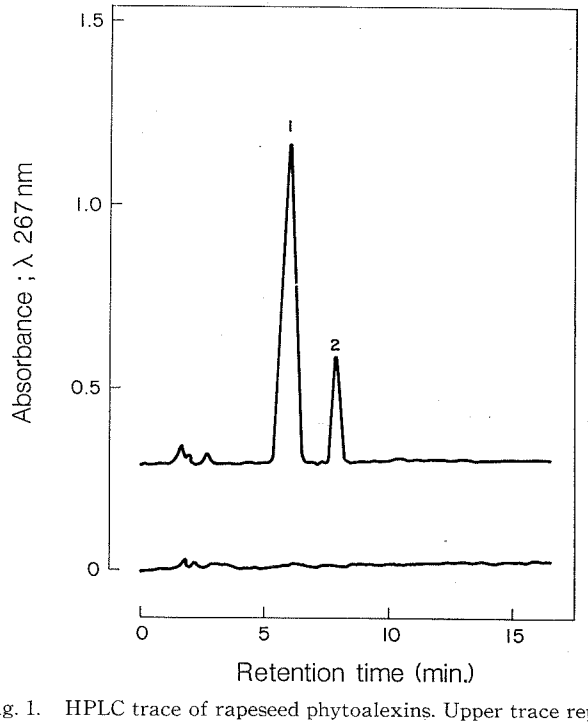

Fig. 1. HPLC trace of rapeseed phytoalexins. Upper trace represents analysis of stem tissue extract from tissues challenged with a non-aggressive isolate of *Leptosphaeria maculans*. Lower trace represents sterile water treated control tissue extract. HPLC solvent used: a mixture of acetonitrile and deionised water (70: 30; v/v). Flow rate 3.0 ml/minute. Column: Ultramax 5C 18 (25×1.0 cm; i. d.). 1, Methoxybrassinin; 2, Cyclobrassinin.

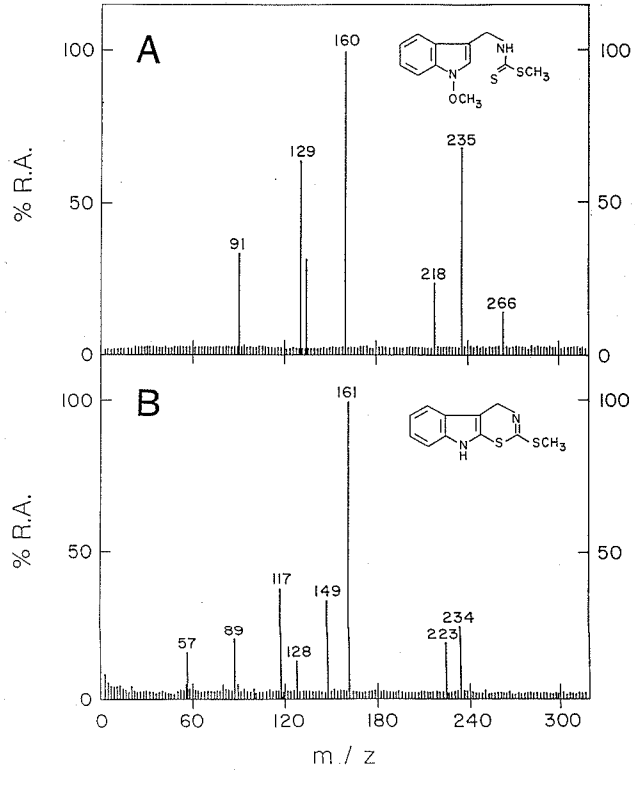

Fig. 2. Mass spectra of purified methoxybrassinin and cyclobrassinin, A: Methoxybrassinin, B: Cyclobrassinin.

ylene group is further connected to the >NH to give the final structure. All the above UV, NMR and mass spectral analysis data agree with methoxybrassinin, a phytoalexin described from *Brassica rapa* [Fig. 2] established through comparison of the spectral data to that of an authentic methoxybrassinin.

Compound 2 with HPLC retention time of 7.6 min [Fig. 1] was obtained as white crystalline powder from aqueous methanol. On mass spectral analysis it gave a molecular ion m/z 234 ($C_{11}H_{10}N_2S_2$ requires 234.021, found 234.033) with fragment ions m/z (rel. int.) 223 (18), 161 (100), 149 (23) 128 (10), 117 (43), 101 (9), 89 (12), 73 (14) and 57 (12). Methanolic solution of the compound gave absorbance maxima λ_{max}^{MeOH} : nm (log ϵ) 220 (4.29), 270 (4.18) and a shoulder at 297 (3.42).

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of the compound indicated the presence of one thiomethyl (-SMe) by a singlet at δ 2.62 (3H) one methylene group by doublet at δ 4.52 and δ 4.69 (each 1H, d, J=15.1 Hz) and one >NH was confirmed by a broad singlet at δ 8.69 (5, exchangeable with D_2O). Irradiation of the >NH proton (δ 8.69) caused nuclear Overhauser enhancement (NOE) of an aromatic proton signal at δ 6.92 (1H, dd, J=7.8, 1.5 Hz, 7-H). Consecutive decoupling experiments assigned three other aromatic protons (δ 7.26, 1H, ddd, J=7.8, 7.8, 1.5 Hz, 6-H; δ 7.10, 1H, ddd, J=6.8, 7.8 and 1.0 Hz 5-H and δ 7.36, 1H, dd, J=6.8, 1.0 Hz, 4-H). Based on UV, NMR and mass spectral analysis the structure assigned to 2 was identical to a cyclobrassinin phytoalexin reported earlier (Takasugi *et al.*, 1986). The final structure [Fig. 2] was assigned by comparing the spectral data to that of an authentic cyclobrassinin.

Table 1. *Abiotic elicitation of phytoalexins in stem and leaf tissues of* ***Brassica juncea*** *cv. Cutlass*

Values represent means of 3 replicates and an quoted as ± standard error. Values in parentheses are phytoalexin values from leaves and values outside parentheses from stem tissues. No phytoalexins were found in sterile water treated controls.

Treatment	Methoxybrassinin (μg g^{-1} fr. wt.)	Cyclobrassinin (μg g^{-1} fr. wt.)
$AgNO_3$ (10^{-3}M)	191.48±7.6 (128.16±5.7)	146.70±5.8 (89.82±4.9)
$CuCl_2$ (10^{-2}M)	118.56±3.8 (112.30±5.9)	78.40±6.9 (72.38±5.1)
$HgCl_2$ (10^{-3}M)	130.56±7.4 (46.90±6.5)	80.92±5.8 (26.84±5.1)
$Pb(NO_3)_2$ (10^{-3}M)	36.47±3.9 (12.19±4.26)	17.66±3.4 (-)
$Ni(NO_3)_2$ (10^{-2}M)	42.58±5.6 (18.60±2.9)	21.77±3.9 (-)
$ZnSO_4$ (10^{-2}M)	50.18±3.6 (26.55±4.1)	28.96±4.8 (9.60±2.01)
UV+$AgNO_3$ (10^{-3}M)	56.39±4.8 (18.44±5.6)	29.46±5.3 (-)
$AgNO_3$ (10^{-3}M) +$Pb(NO_3)_2$ (10^{-3}M)	190.98±6.8 (116.30±7.9)	122.94±4.8 (90.56±3.9)

Effectiveness of Elicitors

The droplet technique was used to test the effectiveness of various elicitors. Heavy metal salts varied greatly in their effectiveness for inducing methoxy-

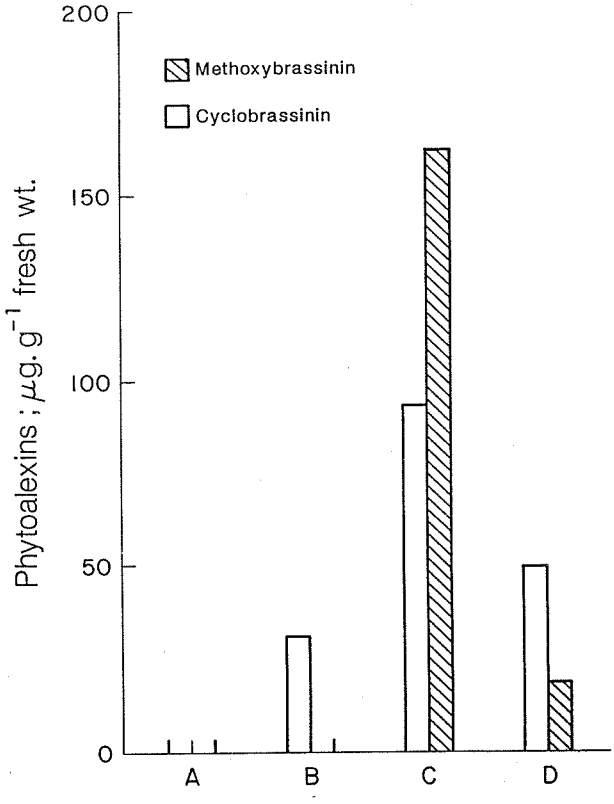

Fig. 3. Phytoalexin accumulation in *B. juncea* cv. Cutlass in response to aggressive and non-aggressive isolates of *Leptosphaeria maculans*. A : Sterile water treated control. B : Stem tissues challenged with an aggressive isolate of *L. maculans*. C : Stem tissues challenged with a non-aggressive isolate of *L. maculans*. D : Stem tissues challenged with a mixture of both aggressive and non-aggressive isolates of *L. maculans*.

brassinin and cyclobrassinin accumulation in *Brassica* leaves. Various concentrations of $Ni(NO_3)_2$, $ZnSO_4$, $Pb(NO_3)_2$ or $HgCl_2$ induced small quantities of methoxybrassinin and cyclobrassinin in leaf tissues following these treatments. However, cupric chloride (20 mM) treatment accumulated more of both methoxybrassinin and cyclobrassinin. Highest concentrations of methoxybrassinin and cyclobrassinin were recorded in the tissues following treatment with silver nitrate (10^{-3} M) whereas, tissues inoculated with sterile distilled water did not accumulate either of these phytoalexins. Simultaneous application of $AgNO_3$ and $Pb(NO_3)_2$, both 10^{-3} M, induced similar phytoalexin accumulation as 10^{-3} M $AgNO_3$ alone. UV-irradiation, immediately prior to the treatment of 10^{-5} M $AgNO_3$, significantly reduced phytoalexin accumulation induced by $AgNO_3$ alone [Table 1].

Both methoxybrassinin and cyclobrassinin accumulated in leaf tissues inoculated with a non-aggressive isolate of *L. maculans* whereas only cyclobrassinin was found to accumulate when challenged with an aggressive isolate [Fig. 3]. The aggressive isolate suppressed accumulation of phytoalexins either by secret-

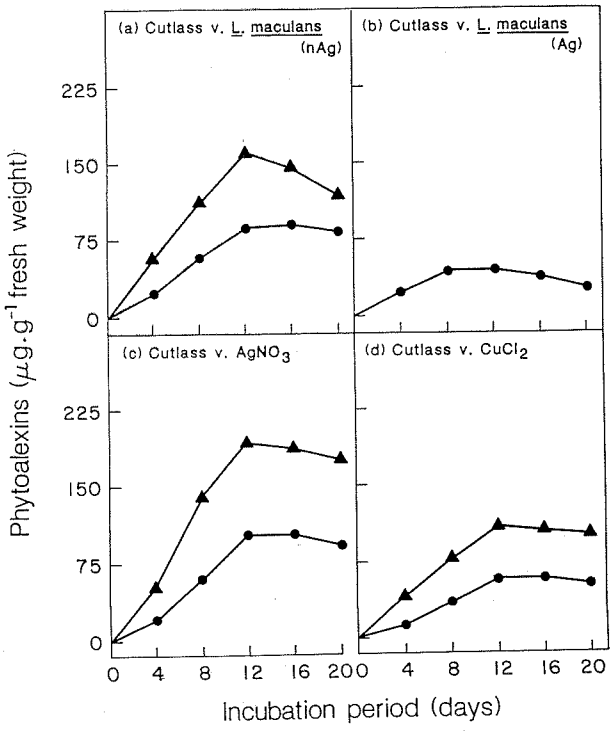

Fig. 4. Time course studies on phytoalexin accumulation by *B. juncea* cv. Cutlass stem tissues

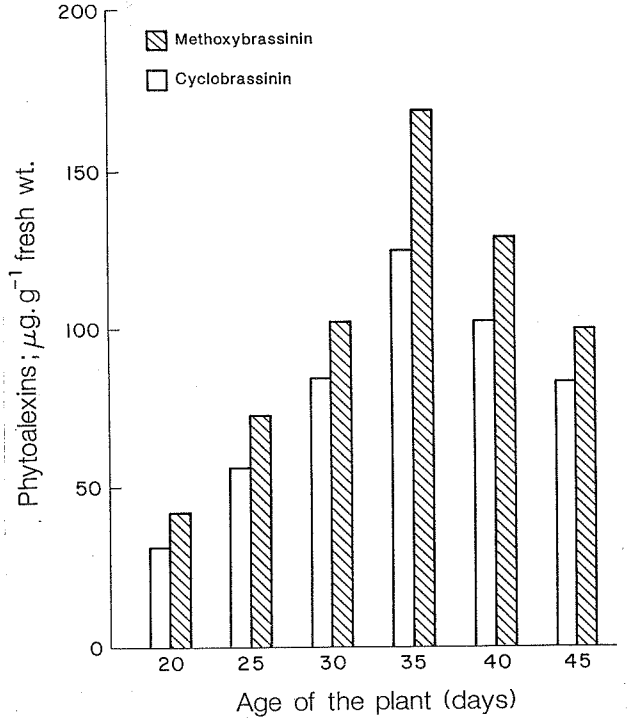

Fig. 5. Effect of age of the *B. juncea* cv. Cutlass plant on phytoalexin accumulation in response to inoculation with a non-aggressive isolate of *Leptosphaeria maculans*.

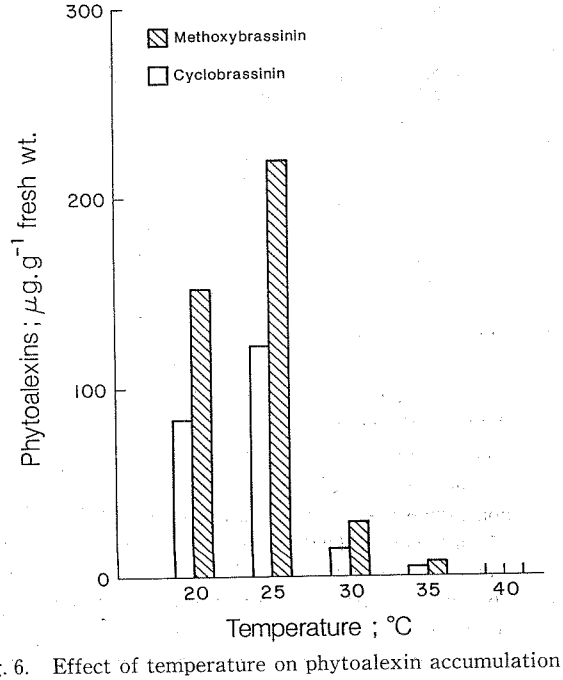

Fig. 6. Effect of temperature on phytoalexin accumulation by *B. juncea* cv. Cutlass in response to inoculation with a non-aggressive isolate of *Leptosphaeria maculans*.

ing a suppressor molecule or metabolizing the accumulated phytoalexins. Treatment with $AgNO_3$ was significantly more effective than inoculation with either non-aggressive or aggressive isolates of *L. maculans* [Fig. 4]. Generally higher levels of phytoalexins accumulated in stem tissues than in leaf tissue of *B. juncea* cv. Cutlass [Table 1]. For example, $HgCl_2$ treatment induced more phytoalexin accumulation in stem slices than leaf tissues [Table 1]. However, with $CuCl_2$ treatment no significant differences in the amount of phytoalexins in stem or leaf tissue occurred.

Effect of age and incubation temperature on phytoalexin accumulation. Leaves from 35 days old plants accumulated more phytoalexins in all the treatments as compared to tissues from 20, 25, 30, 40, or 45 days old plants [Fig. 5]. Incubation of leaf or stem tissues at elevated temperatures of 30, 35 and 40°C led to decline in phytoalexin accumulation. Maximum amounts for both were recorded at 25°C [Fig. 6].

Brassica juncea cv. Cutlass accumulated more phytoalexins both in stem and leaf tissues when challenged with a non-aggressive isolate of *L. maculans* as compared to *B. napus* cv. UM2377 and *B. rapa* cv. Tobin [Fig. 7]. Susceptible germ lines accumulated low amounts of both methoxybrassinin and cyclobrassinin [Table 2].

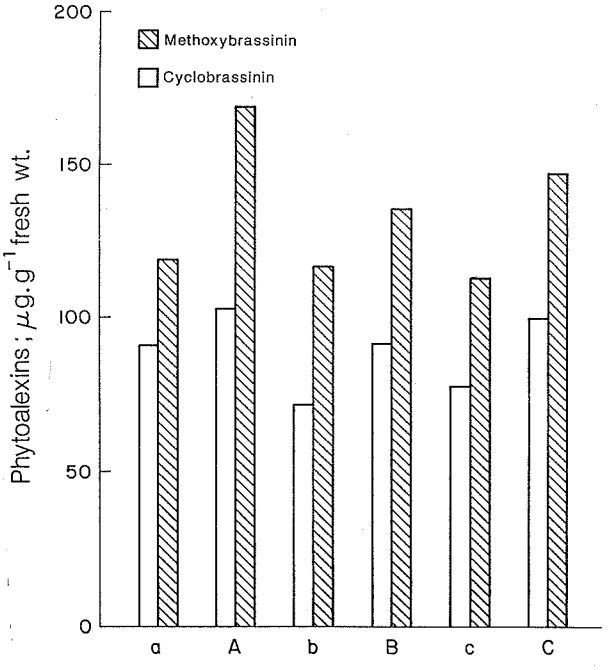

Fig. 7. Phytoalexin accumulation in oilseed *Brassica* plant tissues. a : leaf tissues (*B. juncea* cv. Cutlass) in response to a non-aggressive isolate of *Leptosphaeria maculans*. A : Stem tissues (*B. juncea* cv. Cutlass) in response to a non-aggressive isolate of *L. maculans*. b : Leaf tissues (*B. napus* cv. UM2377) in response to inoculation with a non-aggressive isolate of *L. maculans*. B : Stem tissues (*B. napus* cv. UM 2377) in response to inoculation with a non-aggressive isolate of *L. maculans*. c : Leaf tissues (*B. rapa* cv. Tobin) in response to inoculation with a non-aggressive isolate of *L. maculans*. C : Stem tissues (*B. rapa* cv. Tobin) in response to inoculation with a non-aggressive isolate of *L. maculans*

Table 2. *Phytoalexin accumulation in different Brassica germ lines in response to challenge with a non-aggressive isolate of* ***Leptosphaeria maculans***.

Values represent the means of 4 replicates and are quoted as ± standard error.

Germ line	Methoxybrassinin ($\mu g\ g^{-1}$ fr. wt.)	Cyclobrassinin ($\mu g\ g^{-1}$ fr. wt.)
A. *Brassica napus*		
Westar	16.8±2.8	8.6±3.1
UM 2410	30.6±4.3	18.4±5.2
UM 2374	76.0±3.2	49.5±4.8
UM 2377	132.4±1.8	104.6±3.7
B. *B. juncea*		
Cutlass	160.6±7.3	104.5±6.8
UM 3329	137.5±4.8	96.6±3.8
C. *B. rapa*		
Tobin	68.7±2.9	30.6±5.8
UM 1472	40.6±3.7	18.1±6.5
UM 1467	48.3±3.2	21.1±2.3
UM 3322	70.8±3.9	32.8±1.4

Discussion

Treatment of leaf tissues with $AgNO_3$ (10^{-3}M) was more effective as an elicitor of methoxybrassinin and cyclobrassinin synthesis as compared to the infection from either aggressive or non-aggressive isolates of *L. maculans*. This could be explained by the fact that growth of either isolate of *L. maculans* was limited whereas tissue browning following treatment with $AgNO_3$ is widespread, indicating that tissue damage is more extensive and that a larger number of cells synthesized methoxybrassinin and cyclobrassinin. UV

treatment of leaf tissues prior to treatment with silver nitrate solution led to decrease in phytoalexin accumulation. The reason for this reduction is not clear. $HgCl_2$ treatment, however, induced more phytoalexin accumulation in stem tissues than leaf tissues [Table 2]. Thus the effectiveness of a chemical agent as a phytoalexin elicitor appears to be specific for a plant species as well as for plant organs. Moreover, it is not necessary that all plant parts should accumulate the same amounts of phytoalexins. Differences in the diffusion of an elicitor into various tissues and the sensitivity of cells to an elicitor may account for these variations.

It is apparent from the experimental findings that the highly resistant *B. juncea* lines accumulate more phytoalexins as compared to less resistant *B. napus* and *B. rapa* germ lines [Fig. 7]. The reason for this differential response is yet to be elucidated.

Accumulation of phytoalexins is influenced by the age of the plant tissues and temperature. Our experimental observations indicate that plant tissues from 35 days old plants accumulated more phytoalexins as compared to those from 20, 25 and 40 days old plants. Incubation of plant tissues at elevated temperatures of 30, 35 and 40°C led to drastic reduction in phytoalexin accumulation. Our results are further strengthened by similar reports (Bhattacharya and Ward, 1987; Keen, 1971) that heat treatment of soybean hypocotyl tissues inoculated with race 3 of *Phytophthora megasperma* led to significant reduction in phytoalexin synthesis thus rendering the resistant reaction into a susceptible reaction.

According to Hadwiger and Schwochau (1969) all the described inducers of phytoalexins, e.g., pisatin synthesis, have the potential to change the conformation of double stranded DNA and, thus to activate genes. Some compounds that intercalate into DNA, such as unsubstituted acridine, however, do not induce pisatin production, whereas heavy metal ions have a high affinity for cysteinyl and histidyl side chains of proteins and porphyrins and interact with phosphoryl groups of cellular membranes. The mechanism of action of elicitors in *Brassica* spp. is yet to be understood.

Experiments with *Phaseolus vulgaris* indicate a close association between death of plant cells and the accumulation of phytoalexins in response to abiotic elicitors, biotic elicitors, micro-organisms and viruses. Dead tissue caused the formation of phytoalexins when it was in contact with living cells and extracts of both living and dead tissues were effective elicitors. It is proposed that the accumulation of isoflavonoid phytoalexins represents secondary metabolism mediated through events subsequent to cell injury, i.e., the release of a constitutive elicitor. Whether similar events can explain the accumulation of phytoalexins in *Brassica* tissues is not fully understood. However, analogous effects have been obtained with other legumes, *Vitis vinifers* and *Solanum tuberosum* (Hadwiger and Schwochau, 1969; Langcake *el al.*, 1979; Tomiyama and Fukaya, 1975; Yoshikawa, 1978).

Our results suggest that the non-aggressive strain of *L. maculans* induces more phytoalexin accumulation than the aggressive strain in *Brassica* tissues. This difference seems to be detectable only 9-12 days after inoculation. Decreasing quantity of accumulated phytoalexins in the stem sections of *Brassica* spp. results from a metabolism of methoxybrassinin by either the plant or the aggressive isolate of the pathogen, i.e., *L. maculans*. The difference in the phytoalexins observed could be due to i) a greater elicitation of methoxybrassinin and cyclobrassinin by the non-aggressive isolate, and ii) a greater suppression of methoxybrassinin by the aggresive isolate. Furthermore, a greater elicitation of methoxybrassin and cyclobrassinin accumulation by the non-aggressive isolate may result from a higher release of elicitor(s), a release of more efficient elicitor(s), or a combination of both. The present study suggests that an aggressive isolate counteracts the effect of the non-aggressive isolate on methoxybrassinin accumulation. The lower level of cyclobrassinin again suggests suppression of phytoalexin synthesis by the aggressive isolate. The combination of aggressive and non-aggressive isolates of *L. maculans* led to the lower level of methoxybrassinin and cyclobrassinin.

Prior to this investigation, the only compounds described as phytoalexins in crucifers were methoxybrassinin, brassinin and cyclobrassinin from leaves of *B. rapa* cv Chinese Cabbage inoculated with *Pseudomonas cichorii* (Takasugi *et al.*, 1986). These compounds do not seem to be hydrolysed products of indole glucosinolates since none was detected in the extract following enzymic hydrolysis. Attempts to correlate the role of these phytoalexins and indole

glucosinolate content in rapeseed for disease resistance are in progress.

Acknowledgments. We would like to thank Dr. M. Takasugi, Department of Chemistry, Hokkaido University, Sapporo, Japan for supplying authentic samples of methoxybrassinin, cyclobrassinin and isoprenylated genistein. The financial support of Continental Grain Company Limited and the National Research Council of Canada IRAP programme is greatly appreciated. Special thanks to Lillian Buckler for typing the manuscript.

Literature Cited

Bailey, J.A. and R.S. Burden. 1973. Biochemical changes and phytoalexin accumulation in *Phaseolus vulgaris* following cellular browning caused by tobacco necrosis virus. Physiol. Plant Pathol. **3:** 171-176

Bhattacharya, M.K. and E.W.B. Ward. 1987. Temperature-induced susceptibility of soybeans to *Phytophthora megasperma* v. *sojae*: Phenylalanine ammonia lyase and glyceollin in the host; growth and glyceollin I sensitivity of the pathogen. Physiol. Mol. Plant Pathol. **31**: 407-419.

Conn, K.L., J.P. Tewari, and J.S. Dahiya. 1988. Resistance to *Alternaria brassicae* and phytoalexin elicitation in rapeseed and other crucifers. Plant Science **56**: 21-25.

Darvill, A.G. and P. Albersheim. 1984. Phytoalexins and their elicitors. Ann. Rev. Plant Physiol. **35**: 243-275.

Hadwiger, L.A. and M.E. Schwochau. 1971. Specificity of deoxyribonucleic acid intercalating compounds in the control of phenylalanine ammonia lyase and pisatin levels. Plant Physiol. **47**: 346-351.

Hadwiger, L.A. and M.E. Schwochau. 1969. Host-resistance response: an induction hypothesis. Phytopathology **59**: 223-227.

Hargreaves, J.A. 1979. Investigation into the mechanism of mercuric chloride stimulated phytoalexin accumulation in *Phaseolus vulgaris* and *Pisum sativum*. Physiol. Plant Pathol. **15**: 279-287.

Ingham, J.L. 1982. Phytoalexins from the Leguminosae. *In* J. A. Bailey and J.W. Mansfield (eds.) Phytoalexins. Blackie and Son, Glasgow London, pp. 289-318.

Keen, N.T. 1971. Hydroxyphaseollin production by soybeans resistant and susceptible to *Phytophthora megasperma* v. *sojae*. Physiol. Plant Pathol. **1**: 265-275.

Langcake, P., C.A. Cornford, and R. J. Pryce. 1979. Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves. Phytochemistry **18**: 1025-1027.

Paxton, J.D. 1981. Phytoalexins – a working redefinition. Phytopathol. Z. **101**: 106-109.

Perrin, D.R. and I.A.M. Cruickshank. 1965. Studies on phytoalexins. VII. Chemical stimulation of pisatin formation in *Pisum sativum* L. Austr. J. Biol. Sci. **18**: 803-816.

Rahe, J.E. and R.M. Arnold. 1975. Injury related phaseollin accumulation in *Phaseolus vulgaris* and its implications with regard to specificity of host-parasite interaction. Can. J. Bot. **53**: 921-927.

Takasugi, M., N. Katsui, and A. Shirata. 1986. Isolation of three novel sulphur containing phytoalexins from the Chinese cabbage; *Brassica rapa* L. ssp. *pekinensis* (Cruciferae). J. Chem. Soc. Chem. Comm. 1986: 1077-1078.

Tomiyama, K. and M. Fukaya. 1975. Accumulation of rishitin in dead potato tuber-tissue following treatment with $HgCl_2$. Ann. Phytopath. Soc. Japan **41**: 418-420.

Van Etten, H.D. and S.G. Pueppke. 1976. Isoflavonoid phytoalexins. *In* J. Friend and D.R. Threlfall (eds.) Biochemical Aspects of Plant-parasite Relationships. Academic Press, pp. 213-269.

Yoshikawa, W. 1978. Diverse modes of action of biotic and abiotic phytoalexin elicitors. Nature **275**: 246-249.

芸苔植物受*Leptosphaeria maculans*感染或非生物誘因而於組織部位堆積植物防禦素

J.S. Dahiya[1] and S.R. Rimmer[2]

[1]ABI-Biotechnology Incorporated, 40 Scurfield Blvd., Winnipeg, Manitoba, R3Y 1G4
[2]Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Leptosphaeria maculans 是引起十字花科植物黑脚病或莖潰瘍之眞菌。芸苔植物如接種此眞菌或受非生物誘因之處理，植物組織會有 methoxybrassinin 和 cyclobrassinin 等植物防禦素(phytoalexin) 之出現。硝酸銀是誘導植物產生植物防禦素之最有效的非生物性因子，其效果比接種眞菌強。當芸苔植物受弱病原性之 *Leptosphaeria maculans* 感染時，methoxybrassinin 和 cyclobrassinin 均會出現，但受強病原性之菌株感染時，則只產生 cyclobrassinin。植物中具抗病性之遺傳系 (germ lines) 會比感病性者堆積更多的植物防禦素。此外，植物防禦素之含量也受植株年齡、培養溫度，以及接種方法之影響。