RESISTANCE TO *ALTERNARIA BRASSICAE* IN CRUCIFERS

J.P. Tewari, K.L. Conn and J.S. Dahiya
Department of Plant Science, University of Alberta,
Edmonton, Alberta, Canada T6G 2P5

Introduction

Alternaria brassicae is a necrotroph that causes the blackspot of rapeseed. The disease is economically important worldwide. The available information on host-parasite interactions in this disease is scanty. All cultivars of rapeseed are susceptible to *A. brassicae* to various degrees and show typical lesions consisting of necrotic centers surrounded by chlorotic areas. *Brassica napus* is less susceptible than *B. campestris* which is, in part, due to the presence of greater amounts of epicuticular wax in *B. napus* (Conn, 1986; Conn *et al.*, 1984).

Many vegetable, oleiferous and wild crucifers were screened to locate sources of resistance to *A. brassicae*. This paper reports on resistance found in *Eruca sativa*, *Camelina sativa* and *Capsella bursa-pastoris*.

In plants, sources of resistance to pathogens can be due to many factors. One general source in many plants is the production of phytoalexins (Ersek and Király, 1986). Phytoalexins have been defined as "low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" (Paxton, 1981). This paper will also report on preliminary research that has indicated the elicitation of phytoalexin-type compounds in rapeseed and *C. sativa* in different concentrations, which may explain their differential disease susceptibility. To this point there appears to have been only one previous report on isolation

of phytoalexins from cruciferous plants (Takasugi et al., 1986). These authors found that sulphur-containing phytoalexins were produced in Chinese cabbage (B. campestris ssp. pekinensis) when inoculated with Pseudomonas cichorii or Erwinia carotovora.

Materials and Methods

Plants used in this study were grown under greenhouse conditions. Four canola-type cultivars of rapeseed used in this study included Candle, Tobin (B. campestris), Altex and Westar (B. napus). The other crucifers used were accessions of B. rapa, E. sativa, C. sativa, and C. bursa-pastoris.

Alternaria brassicae was grown on V8 juice medium containing rose bengal (Degenhardt et al., 1974) at 25°C in dark. Conidia were washed off the plates with distilled water, centrifuged, washed twice and resuspended in distilled water.

To screen leaves for their susceptibility to A. brassicae, drops of conidial suspension were placed on leaves kept in a moist chamber and incubated for 4-5 days at room temperature in dark. Drops of distilled water were placed on leaves used as controls. To determine the extent of germination of the conidia, pieces of leaves were cut out, stained with lactophenol cotton blue and observed under a microscope.

To test for the production of phytoalexins, the drops of inoculum were removed, the lesions cut out using cork borers and weighed. If no lesions formed, the areas covered by the drops of inoculum were cut out. In some cases the drops of inoculum were kept separate from the leaf material and in others they were pooled together before extraction. Extraction was done in 70% aqueous methanol (v/v) for 3-5 min using a facilitated diffusion technique (Keen, 1978). The extracts were filtered, evaporated to dryness at ~40°C and redissolved in methanol. These were spotted on thin layer chromatography (TLC) plates (K5 silica gel, 250μm thickness, Whatman) at ratios of 1:1 or 1:2 between weight

(g) of the leaf tissue extracted and length (cm) of the band spotted on the TLC plate. The plates were then developed in chloroform:methanol (49:1) and observed under UV light to locate fluorescent spots. To determine if these spots had antifungal activity, a bioassay was done on the TLC plates. A thick conidial suspension of a Cladosporium sp. in Czapek's 2x broth was sprayed on the plates and the plates incubated in a humid chamber at room temperature for 3 days in dark. At the end of incubation period, the plates were examined for zones of inhibition indicated by clear areas surrounded by the fungal growth.

Results and Discussion

The accession of B. rapa studied was susceptible, showing the same symptoms as the cultivars of rapeseed studied. Figures 1 and 2 show susceptible reactions on leaves of the rapeseed cultivars Candle and Westar, respectively, 4 days after they had been inoculated with A. brassicae on the right side and distilled water on the left side. These lesions continued to expand under favorable conditions. Eruca sativa, on the other hand, showed restricted necrotic flecks resembling hypersensitive reaction (figs. 3 and 4). Figure 3 shows a leaf of E. sativa 4 days after it had been inoculated with A. brassicae on the right side and distilled water on the left. These flecks appeared after 1 to 2 days but did not grow in size, even after 5 days (fig. 4). No chlorosis resulted around these necrotic flecks. Camelina sativa and C. bursa-pastoris were classed as being the most resistant. On these plants the germination of A. brassicae conidia was reduced and penetration of the leaves usually did not take place. No symptoms occurred on the leaves of these plants. Figures 5 and 6 show leaves of C. sativa and C. bursa-pastoris, respectively, 4 days after they had been inoculated with A. brassicae on the right side and distilled water on the left side.

It has been found that the 4 cultivars of rapeseed and the accessions of B. rapa and C. sativa produce phytoalexin-type compounds in response to A. brassicae (Rf 0.33 on TLC plate). The spot produced by C. sativa was much larger and brighter under UV light than those from the others. This spot was found in both the drops of inoculum withdrawn from the leaves and also the leaf tissue of C. sativa, but not in the controls. The bioassay on TLC plate showed this spot to have antifungal activity. The fact that C. sativa produced much more phytoalexin than the susceptible rapeseed cultivars or an accession of B. rapa, may explain their differential disease susceptibility. The possible elicitation of phytoalexin response in E. sativa and C. bursa-pastoris has still to be determined.

This work has, for the first time, recognized elicitation of phytoalexin response in crucifers when challenged by a fungal pathogen. Also, the results indicate that certain crucifers have high degrees of resistance to A. brassicae. Conventional breeding and biotechnological techniques could be used for transferring this resistance to rapeseed.

Acknowledgement

This work was supported through operating grant nos. A 0491 and 85016 from Natural Sciences and Engineering Research Council of Canada and Agriculture Canada, respectively, to the first author.

References

Conn, K.L. 1986. Leaf epicuticular wax of canola: ultrastructure, chemistry and interaction with Alternaria brassicae. MSc thesis. Univ. Alberta, Edmonton, Canada. 159pp.

Conn, K.L., Tewari, J.P., and Hadziyev, D. 1984. The role of epicuticular wax in canola in resistance to Alternaria brassicae. Phytopathology 74:851 (abstr.).

Degenhardt, K.J., Skoropad, W.P., and Kondra, Z.P. 1974. Effects of Alternaria blackspot on yield, oil content and protein content of rapeseed. Can. J. Plant Sci. 54:795-799.

Ersek, T., and Király, Z. 1986. Phytoalexins: Warding-off compounds in plants? Physiol. Plantarum 68:343-346.

Keen, N.T. 1978. Phytoalexins: efficient extraction from leaves by a facilitated diffusion technique. Phytopathology 68:1237-1239.

Paxton, J.D. 1981. Phytoalexins - a working redefinition. Phytopathology 101:106-109.

Takasugi, M., Katsui, N., and Shirata, A. 1986. Isolation of three novel sulphur-containing phytoalexins from the Chinese cabbage Brassica campestris L. ssp. pekinensis (Cruciferae). J. Chem. Soc., Chem. Commun. 14:1077-1078.

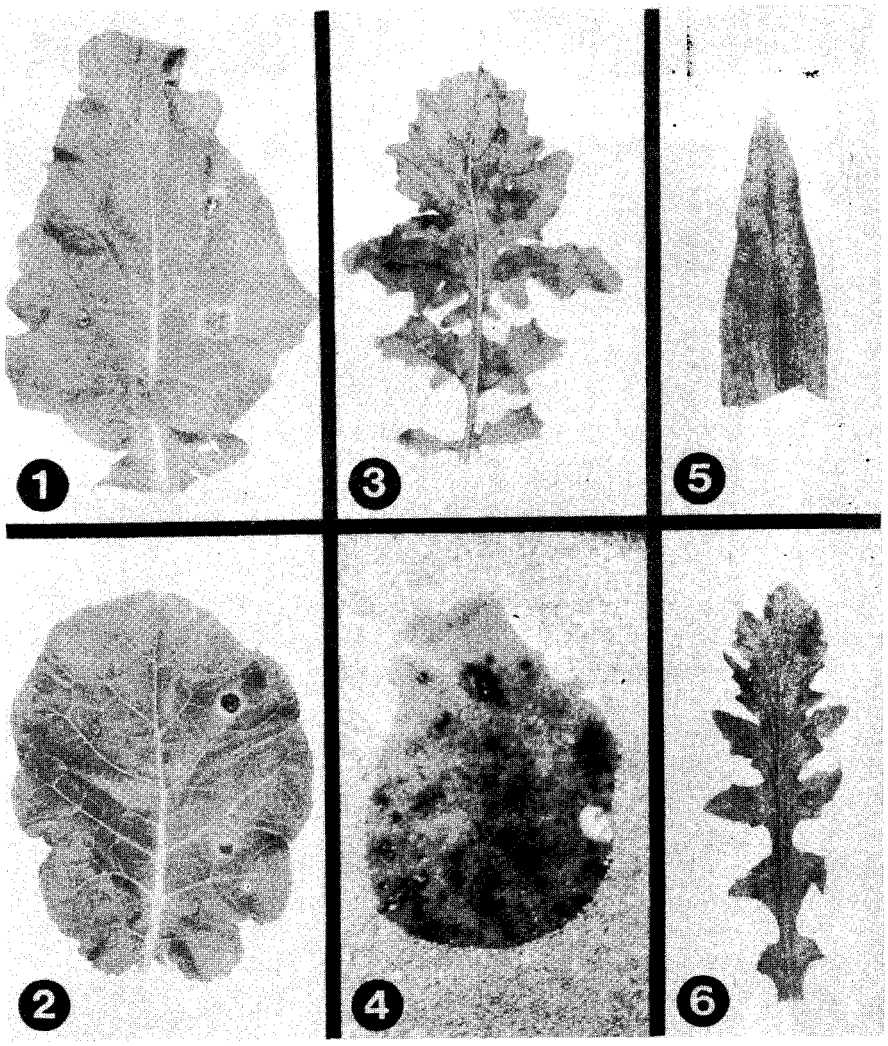
Legend

Figs. 1-6. Leaves of various crucifers after inoculation with A. brassicae on the right side and distilled water on the left. In all cases incubation was for 4 days except for the specimen in Fig. 4 which was incubated for 5 days.

Figs. 1 and 2. Susceptible reactions on the rapeseed cultivars Candle (B. campestris) and Westar (B. napus), respectively.

Figs. 3 and 4. Hypersensitive reaction on Eruca sativa (fig. 4, 8x).

Figs. 5 and 6. Resistant response on Camelina sativa and Capsella bursa-pastoris, respectively.


1
3
5
2
4
6