# PHYTOTOXICITY OF THE CULTURE FILTRATES OF ALTERNARIA SPECIES

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## INTRODUCTION

Alternaria species commonly produce phytotoxins which play a causative role in plant disease (Ueno 1988).

Alternaria brassicae (Berk.) Sacc. and A. raphani Groves & Skolko cause alternaria black spot disease on oilseed brassicas, and are frequently of economic importance on canola and mustard crops in western Canada. Isolates of A. brassicae produce the host-specific phytotoxin destruxin B (Peña-Rodriguez 1985; Bains and Tewari 1987). The culture filtrate of A. raphani contains two groups of phytotoxins (Degenhardt 1977) which remain uncharacterized.

Alternaria brassicicola (Schw.) Wiltshire is an important pathogen of vegetable brassicas. Gloer et al. (1988) isolated a compound with antibiotic properties, brassicicolin A, from the culture filtrate of this species; preliminary research in our laboratory has shown that brassicicolin A is a non-host-specific phytotoxin (Bansal and Séguin-Swartz, unpublished).

Alternaria saponariae (Pk.) Neerg. causes spots on leaves and nodes of cow cockle (Saponaria vaccaria L.), a noxious weed in western Canada. Alternaria gypsophilae Neerg. is a pathogen of baby's breath (Gypsophila paniculata L.) which has become weedy in certain parts of Saskatchewan. Alternaria resedae Neerg. infects the common garden ornamental mignonette (Reseda odorata L.). There is no information on the production of phytotoxins by A. saponariae, A. gypsophilae and A. resedae. Alternaria resedae and A. brassicicola may belong to a natural taxonomic group (Joly 1959) which would likely include A. saponariae.

The purpose of the present study was to assess the phytotoxicity of the culture filtrates of the six Alternaria species grown under various conditions and obtain preliminary information on the production of phytotoxic compound(s).

## MATERIALS AND METHODS

### Fungal isolates

A single isolate of each Alternaria species was used in the study. The isolates were collected from various locations in Saskatchewan. Isolates were single-spored and maintained on V-8 juice agar (200 mL V-8 juice, 800 mL distilled water, 0.75 g $CaCO_3$, 15 g Difco agar) amended with 40 mg/L Rose Bengal and 100 mg/L streptomycin sulfate in 9 cm-diam petri plates, at 20-24° C (12 h photoperiod). Conidiospore suspensions ($10^5$ spores/mL) were prepared aseptically from 14 day-old cultures.

### Preparation of culture media and filtrates

Four liquid culture media were used. Medium 1: minimal medium (Tinline et al. 1960) supplemented with 100 µg/L of thiamine and containing 15 g/L glucose as the carbon source and

3.12 g/L potassium nitrate as the major nitrogen source; medium 2: medium 1 with 15 g/L starch in lieu of glucose; medium 3: medium 1 with 2.35 g/L ammonium succinate in lieu of potassium nitrate; medium 4: medium 1 with 15 g/L starch and 2.35 g/L ammonium succinate in lieu of glucose and potassium nitrate, respectively. The culture media (200 mL/500 mL flask) were autoclaved 20 min at 121°C and 115 kpa.

After cooling, each culture flask was inoculated with 2 mL of conidiospore suspension ($10^5$ spores/mL). The isolates were grown in stationary cultures in the four culture media and at one of three temperatures (20° C, 25° C, 27° C) for 21 days. The cultures were filtered through glass wool and paper (Whatman No. 1) and the filtrates were stored frozen at -20° C.

Plant material and phytotoxicity tests

The plant species used were: Brassica rapa L. yellow sarson 'R500' (turnip rape), B. napus L. 'Westar' (oilseed rape), B. juncea (L.) Czern. 'Cutlass' (mustard), B. carinata Braun 'S67' (Ethiopian mustard), B. nigra L. (black mustard), Sinapis alba L. 'Ochre' (white mustard), Saponaria vaccaria L. (cow cockle), Linum usitatissimum L. 'Vimy' (flax), Hordeum vulgare L. 'Harrington' (barley), Triticum aestivum L. 'Kenyon' (wheat), and Avena sativa L. 'Calibre' (oat). Plants were grown under controlled conditions (20/18° C day/night temperature, 18 h photoperiod). The cotyledons of ten day-old seedlings (first leaf for the cereals) were wounded with a No. 1 entomological needle and each wound was inoculated with 10 µL of raw filtrate. The phytotoxicity of the filtrates was assessed after 7 days using the following scale: -, non toxic (no lesion); +, slightly toxic (lesion diameter: 1-1.5 mm); ++, mildly toxic (1.5-2 mm); +++, toxic (3-5 mm); ++++, highly toxic (≥6 mm). Autoclaved, uninoculated culture media incubated at 20° C, 25° C, or 27° C were used for the control treatments.

## RESULTS AND DISCUSSION

The phytotoxicity of the culture filtrates from each combination of species, culture medium and temperature was tested on cotyledons of B. rapa yellow sarson (Table 1), which is susceptible to A. brassicae (Bains and Tewari 1987; Bansal et al. 1990) and to its toxin, destruxin B (Bains and Tewari 1987).

The culture filtrates of A. brassicae and A. brassicicola were toxic or highly toxic in all 12 combinations of culture medium and incubation temperature; the culture filtrates of A. raphani, however, had a much lower toxicity. A similar difference in phytotoxicity between the two black spot pathogens was observed by Degenhardt (1977). The production of phytotoxic compounds did not appear to be influenced by the carbon or nitrogen source of the culture medium or by the incubation temperature, factors which have been shown to affect growth in these species (Taber et al. 1968).

The culture filtrate of A. saponariae was toxic when the fungus was grown in medium 3 at 25° C, but showed little or no toxicity in all other combinations of culture medium and incubation temperature. For this pathogen, there appears to be an effect of the culture environment on the production of phytotoxic compound(s). The culture filtrates of A. gypsophilae were slightly or mildly (medium 3, 25° C) toxic to rapeseed cotyledons; the phytotoxicity of the filtrates was similar to

Table 1. Phytotoxicity of the culture filtrates of Alternaria species on cotyledons of Brassica rapa yellow sarson 'R500'

| Alternaria species | Medium 1 | | | Medium 2 | | | Medium 3 | | | Medium 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20° | 25° | 27° | 20° | 25° | 27° | 20° | 25° | 27° | 20° | 25° | 27° |
| A. brassicae | +++ | +++ | +++ | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++++ |
| A. brassicicola | +++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | +++ | ++++ |
| A. raphani | + | + | ++ | + | ++ | + | + | ++ | + | + | + | + |
| A. saponariae | - | - | + | - | - | - | - | +++ | + | - | + | - |
| A. gypsophilae | + | + | + | - | + | + | + | ++ | + | - | + | + |
| A. resedae | +++ | +++ | +++ | +++ | +++ | ++++ | +++ | ++++ | +++ | +++ | +++ | +++ |

- Non toxic; + slightly toxic; ++ mildly toxic; +++ toxic; ++++ highly toxic

that of A. raphani. The culture filtrates of A. resedae were all toxic or highly toxic to rapeseed cotyledons. Control inoculations showed no lesion development.

The filtrates of A. saponariae and A. resedae were selected for further study and their phytotoxicity was compared with that of A. brassicicola. The culture filtrates produced in medium 3 at 25° C were inoculated on eleven plant species (Table 2) as described above. Autoclaved, uninoculated culture medium 3 incubated at 25° C was used in the control treatments.

Control inoculations showed no lesions. The culture filtrate of A. brassicicola was highly toxic to all crucifer species tested with the exception of B. carinata 'S67'. The absence of toxicity symptoms on S67 suggests that the culture filtrate of the isolate that we used contained phytotoxic compounds other than brassicicolin A. Brassicicolin A causes leaf lesions on these crucifers, including S67, at concentrations of $10^{-3}$-$10^{-4}$ M; lower toxin concentrations do not

Table 2. Phytotoxicity of the culture filtrates of Alternaria brassicicola, A. resedae and A. saponariae

| Species | A. brassicicola | A. resedae | A. saponariae |
|---|---|---|---|
| Brassica rapa 'R500' | ++++ | +++ | ++++ |
| B. napus 'Westar' | ++++ | +++ | +++ |
| B. juncea 'Cutlass' | ++++ | +++ | ++++ |
| B. carinata 'S67' | - | - | ++++ |
| B. nigra | ++++ | +++ | +++ |
| Sinapis alba 'Ochre' | ++++ | - | ++++ |
| Saponaria vaccaria | ++++ | - | +++ |
| Linum usitatissimum 'Vimy' | - | - | +++ |
| Hordeum vulgare 'Harrington' | - | - | ++++ |
| Triticum aestivum 'Kenyon' | - | - | ++++ |
| Avena sativa 'Calibre' | - | - | ++++ |

- Non toxic; + slightly toxic; ++ mildly toxic; +++ toxic; ++++ highly toxic

produce lesions on these species (Bansal and Séguin-Swartz, unpublished). The filtrate of A. brassicicola was also highly toxic to cow cockle, but did not affect flax or the cereals.

The culture filtrate of A. resedae was phytotoxic only to the Brassica genotypes with the exception of B. carinata 'S67'. With this cultivar, the filtrate caused the production of callus tissue around the point of inoculation. The broth was extracted with ethyl acetate and the aqueous and organic fractions were bioassayed on cotyledons of B. rapa R500; both fractions were found to be phytotoxic. Further testing of Brassica genotypes is required to obtain more detailed information on the phytotoxicity range of the compound(s).

The culture filtrate of A. saponariae was toxic or highly toxic to all species. Freeze-dried filtrate was dissolved in methanol-water (75:25) and analyzed by reversed-phase HPLC. The analysis revealed a compound with a retention time of ca. 9.0 min. Preliminary phytotoxicity tests with the host genotype, cowcockle, indicated that the compound was phytotoxic.

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