Aggressiveness of *Alternaria brassicicola* to Broccoli

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The aggressiveness of 27 isolates of *Alternaria brassicicola* to broccoli plants was investigated in a growth chamber environment. Of the 27 isolates 14 were collected from leaves of broccoli plants which had lesions only on leaves, and 13 were collected from plants on which lesions were restricted to stems and/or flowers. There was no significant difference between aggressiveness of isolates collected from leaves and those collected from stems/flowers. However, isolates originating from leaves were more aggressive on leaves than those collected from stems/flowers. No clear evidence could be found to support the hypothesis that there is tissue specification among isolates collected from different plant parts. Generally, disease index was highest on the leaf blade and lowest on the stem.

KEY WORDS: *Alternaria brassicicola*; broccoli; tissue specification.

For several years Alternaria black spot has been an economically important disease of broccoli, one of Israel’s vegetable export crops. The outbreak of this disease in recent years has greatly restricted the export of processed broccoli and has caused great economic losses. Before the Alternaria black spot outbreak, it was a known common disease of broccoli and other crucifers in Israel, but in broccoli it caused no noticeable reduction in yield or quality, since lesions were found mainly on leaves. However, now the flower head (the harvested part) is heavily infected too. Results of our earlier work showed that the blackspot of broccoli and other crucifer vegetables is caused by at least two *Alternaria* species, *viz.*, *A. brassicicola* and *A. raphani*, with the former being the dominant and more destructive one on brassica vegetables in Israel.

In the field it is often noted that some plants have lesions only on the leaves and others only on the stem and/or flower. This observation led us to a hypothesis that tissue specification might exist among isolates or species of *Alternaria*.

We report here our findings on the aggressiveness of 27 *A. brassicicola* isolates to broccoli plants. Our aim was to investigate the following: (i) Does tissue specification exist among isolates of *A. brassicicola* and is there any difference in aggressiveness between isolates collected from stems or flower heads and those from leaves? (ii) What is the aggressiveness variation in the population of *A. brassicicola*?

From November 1992 to February 1993 diseased tissues with typical Alternaria black spot lesions were collected from commercial broccoli fields (cv. 'Shugon') in the vicinity of Ashdod, Israel. Most plants from which the sample was collected were infected either on the leaves or on the stem/flower. Twenty-seven single conidial spore isolates of *A. brassicicola* (14 from leaves and 13 from stems/flowers) were isolated. The isolates were stored on PDA (potato dextrose agar) slants at 4°C.

In order to estimate the concentration of conidia suspensions quickly and precisely, a spectrophotometric method was developed. A conidial suspension of $2 \times 10^6$ conidia/ml

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was serially diluted into ten different suspensions. The distinction value was measured for each suspension using a spectrophotometer at wavelength = 600 nm. The regression equation calculated by linear regression analysis was:

\[ Y = 1.498 + 156.734 \cdot D \quad (R^2 = 0.99, s^2 = 4.770) \]

where \( Y \) is the conidia concentration (\( x \times 10^4/\text{ml} \)) and \( D \) is the distinction value of the suspension.

For inoculation of broccoli plants, conidial suspensions were prepared by washing the conidia produced by a 10-day culture on a PDA plate at 23°C with distilled water containing 0.1% Tween 20. The conidia suspension was passed through two-layer cheesecloth and allowed to sediment for 10 minutes; the supernatant was discarded and the conidia were resuspended with distilled water containing 0.1% Tween 20. The conidia concentration was estimated spectrometrically by measuring the distinction value at 600 nm wavelength. The conidia concentration was then adjusted to 3 \( \times 10^5/\text{ml} \) for the first experiment and 1 \( \times 10^5/\text{ml} \) for the second.

All experiments were done with the susceptible cultivar Shugon. Three greenhouse-grown broccoli plants at the 6–8-leaf stage were inoculated with each isolate by spraying with spore suspension until runoff. The inoculation of all plants was completed within 20 minutes. The inoculated plants were placed at random in a mist chamber which is equipped with two mist generators (Defensor 505, Defensor AG, Zürich, Switzerland), incubated for 20 h at 18°C in the dark, and then transferred to a growth chamber calibrated to 25°C and illuminated with cool fluorescent light (150 \( \mu \text{E.m}^{-2}\text{sec}^{-1} \), 12 h/day).

Disease development was assessed 8 days after inoculation, using the following index:

0: no symptoms
1: less than 10% infected area
2: 10-25% infected area
3: 26-50% infected area
4: more than 50% infected area

The disease on leaf blades and petioles of three leaves (2nd, 3rd and 4th fully expanded leaves from the top) per plant was evaluated separately. The mean value of the three leaves gave the disease index of the plant. The disease on the stem was assessed according to the same scale. The experiment was repeated twice.

The disease indices on leaf, petiole and stem were analyzed separately, as these data originated from the same plants and are not independent of each other. Because results from the two experiments were similar, the data obtained from them were analyzed together. The data analysis was performed using the ANOVA procedure of the SAS statistical package (8).

To test the effect of plant tissue on the infection of \( A. \) brassicicola, leaf blades (the third and fourth fully expanded leaves from the top) and stems of broccoli plants at the 6–8-leaf stage were inoculated with isolate D12 (from a leaf) in a 5-\( \mu \)l droplet of conidial suspension (5 and \( 10 \times 10^3 \) conidia/ml; ten sites per leaf and five sites per stem). After incubation for 20 h at 18°C in the mist chamber in the dark, plants were transferred to the growth chamber as above. The number of inoculated sites with disease symptoms was counted 10 days after inoculation.

Analysis of variance (ANOVA) showed that the effect of isolate origin is not significant for disease on petiole (\( P = 0.934 \)) and stem (\( P = 0.869 \)), but is significant for disease on leaf (\( P = 0.018 \)) (Table 1). The insignificant effect of isolate origin for disease on stem and petiole indicates that there is no difference in aggressiveness between isolates collected from the leaf and those from the stem/flower. The fact that the effect of experiment is significant is not unexpected, because different conidia concentrations were used in the two experiments.

In plants inoculated with isolates collected from leaves, the mean disease index on the leaf was the highest (2.78), that on the stem was the lowest (1.74), and that on the petiole was intermediate (2.21). In plants inoculated with isolates collected from stems/flowers, the mean disease index was 2.5, 2.2 and 1.7 for leaves, petioles and stems, respectively (the same order of severity as with isolates collected from leaves). No significant