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# *Alternaria* on cruciferous plants. 4. *Alternaria* species on seed of some cruciferous crops and their pathogenicity

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**The incidence of *Alternaria* spp. on seed samples of cruciferous vegetable crops was surveyed between 1990 and 1992. Some commercial seed lots of crucifers which are commonly grown in Japan were infested with *Alternaria* species. Three *Alternaria* species were encountered on the seed samples of *Brassica campestris, B. orelacea,* and *Raphanus sativus*. The most frequently detected species were *A. japonica* and *A. alternata* on *B. campestris, A. brassicicola* on *B. oleracea*, and *A. japonica* and *A. alternata* on *R. sativus*, respectively. *Alternaria brassicae* was not detected in this study. *Alternaria brassicicola* isolates from these crops produced necrotic lesions on all of the crucifer seedlings inoculated, while *A. japonica* induced different reactions in different plants or plant parts depending on isolates used in inoculation tests. In contrast, most isolates of *A. alternata* could not produce necrotic lesions on foliage leaves of crucifers inoculated, although some of them produced clear lesions only on cotyledons. *Alternaria alternata* associated with these cruciferous crop seeds was considered to be an oppotunistic parasite of these crops.**

Key Words——*Alternaria alternata*; *Alternaria brassicicola*; *Alternaria japonica*; pathogenicity; seed pathology.

Fungal pathogens may have an intimate relationship with seeds of their host plant. Since seeds of oilseed rape which are heavily contaminated by *Alternaria brassicae* (Berk.) Sacc. and/or *A. brassicicola* (Scwein.) Wiltshire may rot prior to or shortly after germination (Parry, 1990), many studies have been done concerning *Alternaria* mycoflora on seeds of cruciferous vegetable crops of the world (e.g., Groves and Skolko, 1994; Humpherson-Jones, 1985; Knox-Davis, 1979; Maude and Humpherson-Jones, 1980a, b; Neergaad, 1979; Sivapalan and Browning, 1992). Studies on the fungi associated with seed samples of these crops have been scarcely reported in Japan.

Previously, we surveyed the *Alternaria* species associated with commercial seed samples of Chinese radish (*Raphanus sativus* L. var. *hortensis* Backer), one of the most common root vegetable in Japan (Tohyama et al., 1991). The most frequently encountered species was *A. alternata* (Fr.) Keissler, detected in 14.2-22.0% of seeds in each seed lot tested. This fungus did not show obvious pathogenicity to Chinese radish seedlings. *Alternaria japonica* Yoshii, which is often incorrectly named as *A. raphani* Groves et Skolko (Tohyama and Tsuda, 1990), and *A. brassicicola*, which has rather strong pathogenicity to this crop, were occasionally detected. However, *A. brassicae*, which has sometimes recorded on seed samples of cruciferous crops in Europe and North America, was not detected.

To study in more detail the *Alternaria* mycoflora on commercial seed samples of cruciferous vegetable crops that are commonly grown in Japan, we surveyed seed samples obtained in 1990–1992. Here we report the incidence of *Alternaria* species and their frequency on commercial seed samples of these crops, and their pathogenicity to these crop seedlings.

## Materials and Methods

**Seed samples of cruciferous crops examined for detection of *Alternaria*** The commercial cruciferous seed samples shown in Table 1 were purchased from a seed store in Kusatsu between 1990 and 1992. The year of harvest of each seed sample was given according to the label attached by the seed distributor(s).

**Identification and isolation of *Alternaria* species** Samples of 180–250 seeds were randomly chosen for the survey. They were surface-sterlized with sodium hypochlorite solution (a.i., 1% chloride) for 3 min after rinsing with 70% ethanol for 30 sec. Then the sample seeds were thoroughly washed in sterilized water to remove the residual chloride, and moisture was wipped off with sterilized filter paper. They were placed on V8-juice agar plates (acidified with lactic acid to pH 3.0–3.2 after autoclaving) and incubated at 23–25°C in darkness for 1 wk. The *Alternaria* colonies, if appeared, were checked under a stereo-microscope and the leading edges of them were transferred onto V8-juice agar medium. Identification of *Alternaria* species thus obtained was made by comparison of cultural and morphological characteristics of colony and conidia with those of standard isolates as in previous reports (Tohyama and Tsuda, 1990; Tohyama et al., 1991) and diagnostic keys provided by Ellis (1971)

Table 1. A list of cruciferous crop seeds examined for detection of *Alternaria* spp.

| Scientific name (group) | Common name | The year of harvest | No. of cvs. examined | Cultivars used |
|---|---|---|---|---|
| *Raphanus sativus* (daikon group) | Japanese radish | 1988, '89 | 11 | Uchikigensuke, Ohkura, Hohryo, Hayabutoritokinashi, Taishi, Yamadanezumi, Taibyosohfutori, Natsumino wase 3 goh, Hayafutoriohkura, Fuyudoriohkura, Aokubimiyashigeshirimaru |
| *Brassica oleracea* (capitata group) | Cabbage | 1989 | 11 | Sohshu, Shikidori, Ryokushun, Hatsuharu, Fuyukaze, Nanpoh, Haruhikari 7 goh, Rubiball, Akimachiwase, Y. R. Ranpokanran, Akimachiwase 3 goh |
| *B. oleracea* (botrytis group) | Cauliflower | 1991 | 3 | Snowmarch, Violetqueen, Snow |
| *B. campestris* (pekinensis group) | Chinese cabbage | 1989, '90, '91 | 7 | Musoh, Rokujunichi, Sensho, Daibanseishirona, Ohgatasantosai, Shinazuma, Taibyorokujunichi |
| *B. campestris* (rapifera group) | Turnip | 1989, '90, '91 | 9 | Tamakoshikokabu, Commet, Hakuginkokabu, Tsuyahimekokabu, Shogoindaimarakabu, Honbeniakamarukabu, Yorogiakamarukabu, Siugukina, Hinona |
| *B. campestris* (rapifera group) | Komatsuna, Nozawana | 1989, '90 | 4 | Nozawana, Komatsuna, Kuromisugi, Kaorikomatsuna |
| *B. campestris* (chinensis group) | Taisai | 1991 | 2 | Yukishirotaisai, Nikanmetaisai |
| *B. campestris* (japonica group) | Mibuna, Mizuna | 1991 | 2 | Banseimibuna, Hakukeisensujikyomizuna |
| *B. juncea* (cernua group) | Karashina | 1991 | 1 | Karashina |

and Tohyama (1993).

**Pathogenicity tests on crucifers seedlings** Some of the isolates of the three species of *Alternaria* obtained by the above examination were used for pathogenicity tests against following cultivars of the cruciferous vegetable crops. They are common and representative cultivars of each crop.

Japanese radish; Taibyosofutori. Cabbage; Sohsyu. Chinese cabbage; Taibyorokujunichi. Turnip; Hakuginkokabu, Sugukina and Hinona. Komatsuna; Kromisugi. Taisai; Yukijirotaisai and Nikanmetaisai. Mibuna; Banseimibuna. Karashina; Hakarashina. Cauliflower; Snowmarch.

Surface-sterilized seeds of these cruciferous crops were sown and allowed to grow to the four-leaf stage in unglazed pots at 23-25°C. The inoculum was adjusted to $5 \times 10^4$ to $1 \times 10^5$ conidia/ml of deionized water using conidia produced on V8-juice agar medium. Irradiation of BLB (Matsushita, FL10BL-B, 12 W, Maximum wave length 352 nm) on the colonies was applied, if necessary, for abundant conidial formation. The inoculated plants were kept in a moist chamber overnight, then transferred to an incubator and kept at 23-25°C. After 4-5 days' incubation, the symptoms on cotyledons, foliage leaves and petioles were recorded according to the criteria of disease severities shown on the footnotes of Tables 3-5.

## Results

***Alternaria* species present in commercial seed samples** The incidence of *Alternaria* species and their frequency in different commercial seed samples of cruciferous crops during the survey in 1990-1992 are shown in Table 2. Three *Alternaria* species, *A. japonica, A. brassicicola*, and *A. alternata*, were encountered but *A. brassicae* was not detected in this study. The proportion of infested seeds varied with the crops and cultivars, ranging from 0 to 16.5%.

*Alternaria japonica* occurred on seed samples of *B. campestris*, turnip, nozawana, and komatsuna (rapifera group), mizuna (japonica group), Chinese cabbage (pekinensis group) and *R. sativus*, Japanese radish (daikon group). Of nine cultivars' samples of turnip seed tested, four were infested and one sample contained 11.0% of infested seeds. However, this fungus did not occur in any cultivars' seed samples of *B. oleracea* (cabbage and cauliflower), chinensis group of *B. campestris* (taisai), and *B. juncea* (karashina) in this study.

*Alternaria brassicicola* was found in cultivars' seed samples of *R. sativus, B. oleracea*, and *B. campestris*. It was encountered on more than 30% of seed samples in the former two vegetables and most heavily infected cultivar sample contained 6.0% of infested seeds. Detection was confined to two of seven samples of Chinese cabbage and one out of nine samples of turnip.

*Alternaria alternata* was the most frequently encountered fungus in cruciferous seed samples surveyed, occurring in all except cauliflower and mizuna. The highest incidences of the fungus were found in seed samples of turnip and nozawana, at 16.5 and 16.0%, respectively.

**Pathogenicity of *Alternaria* species on crucifers** The pathogenicity of these *Alternaria* species was determined by inoculation tests of four-leaf-stage seedlings. Arbitrary isolates of three species of *Alternaria, A. brassicicola, A. japonica*, and *A. alternata*, obtained from seeds of 5-6 cruciferous crops, respectively, were inoculated onto seedlings of source crops and other cruciferous crops.

All *A. brassicicola* isolates caused necrotic lesions on all of the seedlings regardless of host species inoculated in this study (Table 3). For example, an isolate from Chinese radish could cause serious disease symptoms on corresponding Chinese radish seedlings and also on the

Table 2. Incidence of *Alternaria* species on commercial seed lots of cruciferous crops.

| Crucifer seeds examined | | Not detected cvs. | Detected cvs. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *A. japonica* | | *A. brassicicola* | | *A. alternata* | |
| Common name | No. of cvs. | | Cvs.[1] | Range[2] | Cvs.[1] | Range[2] | Cvs.[1] | Range[2] |
| Japanese radish | 11 | 5 | 5 | 0.5- 3.0% | 5 | 0.5-6.0% | 5 | 0.5- 3.5% |
| Cabbage | 11 | 4 | 0 | | 7 | 0.6-2.5 | 2 | 0.6- 0.6 |
| Cauliflower | 3 | 2 | 0 | | 1 | 1.3 | 0 | |
| Chinese cabbage | 7 | 3 | 4 | 0.5- 1.5 | 2 | 0.5-1.0 | 3 | 2.0-12.3 |
| Turnip | 9 | 2 | 4 | 0.6-11.0 | 1 | 0.6 | 6 | 0.3-16.5 |
| Komatsuna, Nozawana | 4 | 1 | 3 | 1.0- 2.0 | 1 | 1.0 | 3 | 10.0-16.0 |
| Taisai | 1 | 1 | 0 | | 0 | | 1 | 0.5 |
| Mibuna, Mizuna | 2 | 1 | 1 | 5.3 | 0 | | 0 | |
| Karashina | 1 | 0 | 0 | | 0 | | 1 | 1.3 |

[1] No. of cultivars detected. [2] Range of proportion of infected seeds (%).

other cruciferous crop seedlings. Moreover, there was almost no difference of the disease severity among the isolates used or crops inoculated. Exceptionally, one isolate from komatsuna produced rather milder necrotic lesions both on komatsuna and Japanese radish. In addition, disease severities were rather milder on foliage leaves than on cotyledons in some inoculated seedlings.

*Alternaria japonica* infected and caused disease on all seedlings inoculated (Table 4). The incidence of diseased lesions on the seedlings varied among isolates and with the different combinations of isolates and crops. There was also a difference in the incidence of lesions among plant parts examined. Cotyledons were generally susceptible to this fungus.

Most isolates of *A. alternata* did not cause any symptoms on foliage leaves and petioles of inoculated cruciferous seedlings (Table 5). Exceptionally, 2 of 5 isolates from Chinese cabbage caused a few clear lesions on foliage leaves of Chinese cabbage seedlings and one out of 15 isolates from Chinese radish caused severe symptoms on cotyledons of Chinese radish seedlings. The results indicate that some *A. alternata* isolates probably

Table 3. Results of inoculation tests on cruciferous crop seedlings with *A. brassicicola* isolated from commercial seed lots in Japan.

| Isolated from | No. of isolates tested | Inoculated to | Cultivar(s) inoculated | Disease severity[1] | | |
|---|---|---|---|---|---|---|
| | | | | Cotyledon | Foliage leaf | Petiole |
| Japanese radish | 8 | Japanese radish | Taibyosofutori | +++ | +++ | +++ |
| | 1 | Komatsuna | Kuromisugi | +++ | +++ | +++ |
| | 1 | Chinese cabbage | Taibyorokujunichi | +++ | ++ | +++ |
| | 2 | Taisai[2] | Yukijirotaisai | +++ | +++ | +++ |
| | | | Nikanmetaisai | +++ | +++ | +++ |
| | 1 | Turnip | Sugukina[2] | +++ | +++ | +++ |
| | | | Hinona[2] | +++ | +++ | +++ |
| | 1 | Mibuna[2] | Banseimibuna | ++ | ++ | +++ |
| | 1 | Karashina[2] | Hakarashina | +++ | ++ | +++ |
| Cabbage | 5 | Cabbage | Sohsyu | +++ | +++ | +++ |
| | 1 | Japanese radish | Taibyosofutori | +++ | +++ | +++ |
| Turnip | 1 | Turnip | Hakuginkokabu | +++ | +++ | +++ |
| | | Cabbage | Sohsyu | +++ | +++ | +++ |
| Chinese cabbage | 1 | Chinese cabbage | Taibyorokujunichi | +++ | ++ | +++ |
| | | Japanese radish | Taibyosofutori | +++ | +++ | +++ |
| Komatsuna | 3 | Komatsuna | Kuromisugi | +~+++ | +~+++ | +~+++ |
| | | Japanese radish | Taibyosofutori | +~+++ | +~+++ | +~+++ |

[1] Disease severity was judged by the following criteria. +++: Numerous lesions, some fused together. ++: Many lesions, but not fused. +: Clear lesions. [2] *Alternaria brassicicola* was not detected on these crop seeds.

Table 4. Results of inoculation test on cruciferous crop seedlings with *A. japonica* isolated from commercial seed lots in Japan.

| Isolated from | No. of isolates tested | Inoculated to | Cultivar(s) inoculated | Disease severity[1] | | |
|---|---|---|---|---|---|---|
| | | | | Cotyledon | Foliage leaf | Petiole |
| Japanese radish | 10 | Japanese radish | Taibyosofutori | ++～+++ | ++～+++ | ++～+++ |
| Chinese cabbage | 1 | Japanese radish | Taibyosofutori | +++ | +++ | ++ |
| Turnip (Kokabu) | 3 | Japanese radish | Taibyosofutori | +～+++ | +～+++ | ±～+++ |
| Turnip (Suguki) | 1 | Komatsuna | Kuromisugi | +++ | +++ | +++ |
| | 1 | Turnip | Hinona | +++ | ++ | + |
| | 1 | Chinese cabbage | Taibyorokujunichi | +++ | +++ | ++ |
| | 1 | Cabbage[2] | Sosyu | +++ | ++ | + |
| | 1 | Taisai | Yukijirotaisai | ++ | ++ | + |
| | | | Nikanmetaisai | +++ | +++ | + |
| | 1 | Karashina[2] | Hakarashina | +++ | + | + |
| Komatsuna | 2 | Japanese radish | Taibyosofutori | +++ | +～+++ | +++ |
| | 2 | Komatsuna | Kuromisugi | +～+++ | +～+++ | +～+++ |
| Mibuna | 1 | Chinese cabbage | Taibyorokujunichi | ++ | ++ | + |
| | 1 | Japanese radish | Taibyosofutori | +++ | ++ | ++ |
| | 1 | Cauliflower[2] | Snowmarch | +++ | ++ | ++ |
| | 1 | Mibuna | Banseimibuna | + | + | ++ |
| | 1 | Karashina[2] | Hakarashina | ++ | + | + |

[1] Disease severity was judged by the following criteria. +++: Numerous lesions, some fused together. ++: Many lesions, but not fused. +: Clear lesions. ±: Most leaves healthy, but clear lesions on a few leaves. [2] *Alternaria japonica* was not detected on these crop seeds.

Table 5. Results of inoculation tests on cruciferous crop seedlings with *A. alternata* isolated from commercial seed lots in Japan.

| Isolated from | No. of isolates tested | Inoculated to | Cultivar(s) inoculated | Disease severity[1] | | |
|---|---|---|---|---|---|---|
| | | | | Cotyledon | Foliage leaf | Petiole |
| Japanese radish | 10 | Japanese radish | Taibyosofutori | −～± | − | − |
| | 5 | | | + | − | ± |
| | 1 | | | +++ | − | ± |
| Chinese cabbage | 1 | Chinese cabbage | Taibyorokujunichi | + | − | − |
| | 1 | | | ++ | − | ± |
| | 2 | | | +++ | + | + |
| | 1 | | | +++ | − | + |
| Turnip | 21 | Turnip | Hakuginkokabu | − | − | − |
| Cabbage | 4 | Cabbage | Sohsyu | −～± | − | − |
| | 1 | | | ++ | − | − |
| Komatsuna | 1 | Komatsuna | Kuromisugi | − | − | − |
| | 2 | Japanese radish | Taibyosofutori | + | − | − |
| Karashina | 1 | Karashina | Hakarashina | − | − | − |
| | 1 | | | + | − | − |

[1] Disease severity was judged by the following criteria. +++: Numerous lesions, some fused together. ++: Many lesions, but not fused. +: Clear lesions. ±: Most leaves healthy, but clear lesions on a few leaves. −: Healthy, no lesion was observed.

have an ability to infect only weak organs such as cotyledons of a seedling. Thus, *A. alternata* inhabiting cruciferous plant seeds is considered to be an opportunistic pathogen, as was suggested in our previous report (Tohyama et al., 1991).

## Discussion

Four *Alternaria* species–*A. japonica* (incorrectly named as *A. raphani*), *A. alternata* (frequently treated as *A. tenuis*), *A. brassicicola* (sometimes as *A. oleracea*), and *A. brassicae* have been recorded on seed samples of crucifer crops (Neergaad, 1979). The frequency of detected species, however, varied depending on the crop, the production district, and year, and reporter.

In the current experiments, *A. japonica* was commonly found on the seed samples of rapifera and japonica group vegetable crops of *B. campestris*. Previous workers have scarcely reported the association of this fungus with cruciferous crop seeds. Only Groves and Skolko (1944) described this fungus on seed samples of radish collected in Ontario, Canada, under the name of *A. raphani*. However, the occurrence of this species in crucifer seeds has not been reported by other researchers. This may be due to these works focusing on seeds of *B. oleracea* on which other *Alternaria* species cause more serious disease in the fields (e.g., Maude and Humpherson-Jones,1980a, b; Sivapalan and Browning, 1992). The present results that seeds of *B. oleracea* do not seem to be infected with *A. japonica* agree with these reports.

The incidence of *A. brassicae* in seed samples of cruciferous crops was also reported differently by different workers. Groves and Skolko (1944) showed that *A. brassicae* was uncommon in seed samples of *R. sativus* in Canada. Maude and Humpherson-Jones (1980a) reported that the fungus was also uncommon in commercial seeds harvested in 1976 and 1977, but was frequently detected in *B. oleracea* seeds harvested in 1978 in Britain. A high recovery of *A. brassicicola* and no detection of *A. brassicae* in *B. orelacea* seed samples was noted in Victoria, Australia, by Sivapalan (1992). Humpherson-Jones (1985) reported the results of five years' survey of *Alternaria* species on seed samples of *B. campestris* (turnip) in Britain in 1979–1983. In this crop, *A. brassicae* was frequently detected and *A. brassicicola* followed. However, *A. brassicae* was not detected in any seed samples examined in the present study. It is of interest that the proportion of seeds infested by this fungus was variously reported for seed samples of different origins. We often found the fungus on Chinese radish leaves in the fields (Tohyama et al., 1991) and easily isolated it from infected leaves, but it was difficult to find and isolate the fungus from seed samples (Tohyama et al., 1991). It is presumed that factors such as climatic conditions in the seed producing area, pre- and post-harvest practices affect the incidence of *A. brassicae* on the seed of crucifers. The slow growth of *A. brassicae* and rapid growth of the associated *A. alternata* and other contaminants might be another reason.

Two *Alternaria* species, *A. brassicicola* and *A. japonica* caused necrotic lesions on the seedlings of considerable numbers of cruciferous crops, irrespective of the source of the isolates (Tables 3, 4). However, severities of the disease caused by *A. japonica* were considerably different depending on the isolates and crops used. Since we used only few cultivars of each cruciferous crop as host plant testers, somewhat different results might be obtained on the reaction of crops to *A. japonica* isolates, if different cultivars of the crops would be used.

Most isolates of *A. alternata* did not produce definite symptoms on these crops. However, four isolates from Chinese cabbage bore numerous lesions on cotyledons on Chinese cabbage, and two of them caused a few clear lesions even on foliage leaves. Some isolates from other cruciferous crop seeds bore lesions on cotyledons of seedlings of their origin, but not on foliage leaves. Thus these *A. alternata* isolates share the same opportunistic nature with Chinese radish isolates which have been reported previously (Tohyama et al.,1991).

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## Literature cited

Ellis, M. B. 1971. "Dematiaceous Hyphomycetes," pp. 464–497. CMI, Kew, Surrey.

Grove, J. W. and Skolko, A. J. 1944. Notes on seed-borne fungi II. *Alternaria*. Can. J. Res. C **22**: 217–244.

Humpherson-Jones, F. M. 1985. The incidence of *Alternaria* spp. and *Leptospaeria maculatum* in commercial brassica seed in the United Kingdom. Plant Pathol. (London) **34**: 385–390.

Knox-Davis, P. S. 1979. Relationship between *Alternaria brassicicola* and *Brassica* seeds. Trans. Br. Mycol. Soc. **73**: 235–248.

Maude, R. B. and Humpherson-Jones, F. M. 1980a. Studies on the seed-borne phases of dark leaf spot (*Alternaria brassicicola*) and grey leaf spot (*Alternaria brassicae*) of brassicas. Ann. Appl. Biol. **95**: 311–319.

Maude, R. B. and Humpherson-Jones, F. M. 1980b. The effect of iprodione on the seed-borne phase of *Alternaria brassicicola*. Ann. Appl. Biol. **95**: 321–327.

Neergaad, P. 1979. "Seed Pathology," pp. 203–208. Macmillan Press, London.

Parry, D. 1990. "Plant pathology in agriculture," pp. 26–27. Cambridge Univ. Press, Cambridge.

Sivapalan, A. and Browning, J. W. 1992. Incidence of *Alternaria brassicicola* (Schw.) Wiltsh. on *Brassica oleracea* seeds. Aust. J. Expt'l. Agric. **32**(4): 535–537.

Tohyama, A. 1993. Identification of *Alternaria* species in Japan. J. Antibact. Antifung. Agents **20**: 107–115. (In Japanese.)

Tohyama, A. and Tsuda, M. 1990. *Alternaria* on cruciferous plants 1. Identity of *Alternaria japonica* and *A. raphani*. Trans. Mycol. Soc. Japan **31**: 501–509.

Tohyama, A., Kohmoto, K., Yu, S. H. and Tsuda, M. 1991. *Alternaria* on cruciferous plants 2. *Alternaria* species on seed of Chinese radish and their pathogenicity. Trans. Mycol. Soc. Japan **32**: 247–258.

Tohyama, A., Nishiguchi, Y., Sagasaki, M., Shiihashi, Y. and Tsuda, M. 1992. *Alternaria* leaf spot of Chinese radish caused by *Alternaria brassicicola* (Sch.) Wiltshire. Sci. Rept. Shiga Pref. Junior Coll. No. 42: 20–28. (In Japanese.)