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Occurrence of Eggplant Wilt Caused by *Verticillium dahliae*

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A wilt disease occurred on greenhouse-grown eggplants at Yeojoo, Korea in 1997. The wilted eggplants had leaves with gradual yellowing, interveinal necrosis, and marginal crinkling. Vascular tissues of diseased stems were discolored, turned black, and microsclerotia developed at the base of stems. The disease progressed from lower parts of the plants upward. Fungal isolates from discolored vascular tissues were initially whitish to cream color on potato-dextrose agar (PDA) plates, which later turned black due to the formation of microsclerotia. Conidiophores were erect, hyaline, verticillately branched, and had 3 or 4 phialides arising at each node. Phialides were hyaline, arranged in whorls, and measured as 17.5-32.5 × 2-3 μm. Conidia were hyaline, ellipsoidal to sub-cylindrical, mainly one-celled, and measured as 5-8.8 × 2-4 μm. Conidia were borne in small clusters at the tips of phialides. Microsclerotia formed on PDA plates, and consisted of globular cells that formed irregular masses of various shapes. Chlamydospores were absent. Based on these cultural and morphological characteristics, the fungus was identified as *Verticillium dahliae* Klebahn. Pathogenicity tests by root cutting, root dipping or soil drenching resulted in similar symptoms observed in the naturally infected eggplants. This is the first report on occurrence of Verticillium wilt of eggplant in Korea.

Keywords **:** Cryo-SEM, soil-borne pathogen, *Solanum melongena*, *Verticillium*.

Verticillium spp. are economically important wilt pathogens of many plants. These soil-borne pathogens have a wide host range consisting of more than 200 plant species with worldwide distributions (Agrios, 1997; Isaac, 1967). They cause vascular wilts of vegetables (Dobinson et al., 1996), flowers (Church and McCartney, 1995), perennial ornamentals (Skarmoutsos and Skarmoutsou, 1998), and forest trees (Harada et al., 1997). Two *Verticillium* species, *V. albo-atrum* Reinke and Berthold and *V. dahliae* Kleb., have been reported to cause the wilts of most plants. These fungi induce wilts at lower temperatures than *Fusarium* spp.; moreover, the symptoms develop more slowly and often appear only on the lower or outer part of the plants (Agrios, 1997).

Verticillium wilt of eggplant (*Solanum melongena* L.), caused by *V. dahliae* Klebahn, is a destructive disease of eggplant (Kamal and Saydam, 1970). Symptoms appear usually around flowering, and include unilateral wilting, chlorosis, and defoliation (Elmer and Ferrandino, 1991). The disease may destroy an entire field in highly infested areas with the fungus (Marois et al., 1982). While there have been reports on Verticillium wilt in Korea on sesame (Park, 1967), chrysanthemum (Lee et al., 1991), and tomato (Park et al., 1995), no information has been available on occurrence of Verticillium wilt of eggplant in Korea. The objectives of this study were 1) to identify the pathogen on eggplant, 2) to determine the pathogenicity of the pathogen on eggplant, and 3) to report a new disease in Korea.

Materials and Methods

Disease survey. Disease survey was performed on eggplants grown in greenhouses at Yeojoo, Korea in 1997. A total of 50 eggplants were observed at 20-day intervals in greenhouses from late May to early July in that year. Symptoms were examined either on whole plants or eggplant leaves. Incidence of the diseased plants was recorded and expressed as mean percent of eggplants showing wilt or interveinal chlorosis symptoms. Some eggplants exhibiting the characteristic symptoms of Verticillium wilt were collected for isolation of the causal pathogen.

Pathogen identification. Small stem pieces (about 5 cm long) were cut from the base of the wilted eggplants. The epidermis and the bark were aseptically removed, and cross-sections of the pieces were transferred to water agar plates using a sterilized razor blade. After incubation at 25°C for 5 days, fungal colonies were transferred to potato-dextrose agar (PDA) plates at 25°C under continuous fluorescent light for 4 weeks. Hyphae were removed from plates and examined with a light microscope (Axiophot, Zeiss, Germany) under differential interference contrast mode. For scanning electron microscopy, agar plugs were removed from the PDA plates with a razor blade. The agar plugs were mounted to metal stubs using carbon tapes, and plunged into liquid nitrogen for 10 seconds for cryo-fixation. The specimens were placed in a cryo-chamber of a cryo-transfer system (CT1500, Oxford Instru-

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ments, UK) maintained at -170°C, and transferred to a cold specimen stage of a scanning electron microscope (JSM-5410LV, JEOL, Japan). Sublimation was performed by heating and maintaining the cold specimen stage at -70°C for 3 min. The specimens were then sputter-coated with gold and observed with the scanning electron microscope at 20 kV. The fungus was identified based on cultural and morphological characteristics.

Inoculum preparation. An isolate (VD-9833) was grown on PDA plates at 25°C under continuous fluorescent light for 8 days. Freshly made PDA plates were streaked with the 8-day-old agar plugs, and incubated at 25°C under continuous fluorescent light for 11 days. Conidia of the fungus were harvested by flooding the plates with sterilized distilled water and scraping the surface of the PDA plates using a paintbrush. The resulting suspension was filtered through two layers of cheesecloth to remove mycelial fragments. Inoculum concentration of the suspension was adjusted to 3×10^7 conidia/ml using a hemacytometer.

Pathogenicity tests. Eggplant seedlings were grown in plastic pots filled with loamy sand soil. When plants reached at the 4-leaf stage 4 weeks after planting, three inoculation methods were employed to test the pathogenicity of the isolate: 1) Root cutting (RC) method; The plants were lifted from the pots, and washed free of excessive soil with tap water. Eggplant roots were cut to 3 cm using scissors, dipped in the conidial suspension for 10 min, and then replanted into the same pot. Check plants were also root-trimmed as described above, dipped in distilled water for 10 min, and then replanted into the same pot. 2) Root dipping (RD) method; Eggplant roots were washed with tap water, dipped in the conidial suspension for 10 min, and then replanted into the same pot. Check plants were dipped in distilled water for 10 min and then replanted into the same pot. 3) Soil drenching (SD) method; Each eggplant was inoculated with 15 ml of the conidial suspension, applied around the base of the hypocotyls. Check plants received 15 ml distilled water. The experiment was conducted as a completely randomized design, with six eggplants per treatment.

After 8 weeks of inoculation, 10 stems of randomly selected diseased eggplants were sampled described above to reisolate *V. dahliae*. After incubation at 25°C for 7 days, the fungal colonies were identified based on cultural and morphological characteristics as described above.

Disease assessment. Every leaf of each eggplant was examined until 8 weeks after inoculation. Incidence of diseased seedlings was recorded as mean percent of eggplant with either interveinal chlorosis or marginal crinkling. Incidence of diseased leaves was also determined as mean percent of diseased leaves exhibiting the characteristic symptoms per eggplant. In addition, severity of diseased leaves was expressed as mean percentage of chlorotic areas in leaves showing interveinal chlorosis and marginal crinkling symptoms.

Results

Disease occurrence. Eggplants exhibiting wilt symptoms were found in greenhouses in the survey (Fig. 1A). The wilted plants had leaves with gradual yellowing, interveinal necrosis, and marginal crinkling (Fig. 1B, 1C). Leaves of infected plants withered and dropped off earlier than healthy leaves. The symptoms progressed from lower part of the plant upwards. Vascular stem tissues were discolored, turned black, and microsclerotia developed at the stem base. Incidence of diseased eggplants was 5% on May 23, 1997. Although the incidence increased to 10% on June 13, it remained constant through early July, 1997.

Fungal identification. The fungal cultures were initially whitish to cream color until 2 weeks of incubation. Cultures later turned black due to the formation of microsclerotia after 4 weeks (Fig. 1D). Conidiophores were erect, hyaline, and verticillately branched (Fig. 2A). Conidiophores had 3 to 4 phialides arising at each node. Phialides were hyaline, arranged in whorls, and measured as $17.5\text{-}32.5 \times 2\text{-}3$ μm (Fig. 2B). Conidia were borne in small clusters at the tips of phialides. It appeared that conidia were usually held together in globose to oval drops by mucilaginous substances (Fig. 3A). Conidia were hyaline, ellipsoidal to subcylindrical, mainly one-celled, and measured as $5\text{-}8.8 \times 2\text{-}4$ μm (Fig. 3B). Microsclerotia consisted of globular cells that formed irregular masses (Fig. 3C), and some globular cells often produced germ tubes. Chlamydospores were absent. Based on these cultural and morphological characteristics, the fungal isolate was identified as *Verticillium dahliae* Klebahn (Table 1).

Pathogenicity tests. Symptoms were first observed on lower leaves of each eggplant 3 weeks after inoculation, and were similar to those observed in naturally infected eggplants. Symptoms were not observed on any check plants. Eggplants inoculated by the RC and RD methods exhibited similar patterns of disease development. Every plant inoculated by the RC and RD methods showed chlorotic leaves 4 weeks after inoculation (Fig. 4A), whereas only 67% of the plants showed chlorotic leaves by the SD method. The incidence and the severity of chlorotic leaves of seedlings inoculated by the RC and RD methods were higher than those by the SD method (Fig. 4B, 4C). All isolates obtained from the 10 randomly selected stems were identified as *V. dahliae*.

Discussion

This study demonstrated occurrence of eggplant wilt caused by *Verticillium dahliae*. This is the first report on Verticillium wilt of eggplant in Korea. The symptoms and the causal organism were identical with those reported earlier (Kamal and Saydam, 1970; Kitazawa and Suzui, 1980). The causal organism of Verticillium wilt of eggplant had been previously regarded as *V. albo-atrum* Reinke and Berthold (Cox, 1956; Wollenweber, 1913). However, this has been changed to *V. dahliae* Klebahn, based on the formation of microsclerotia (Richardson, 1933). The relationship

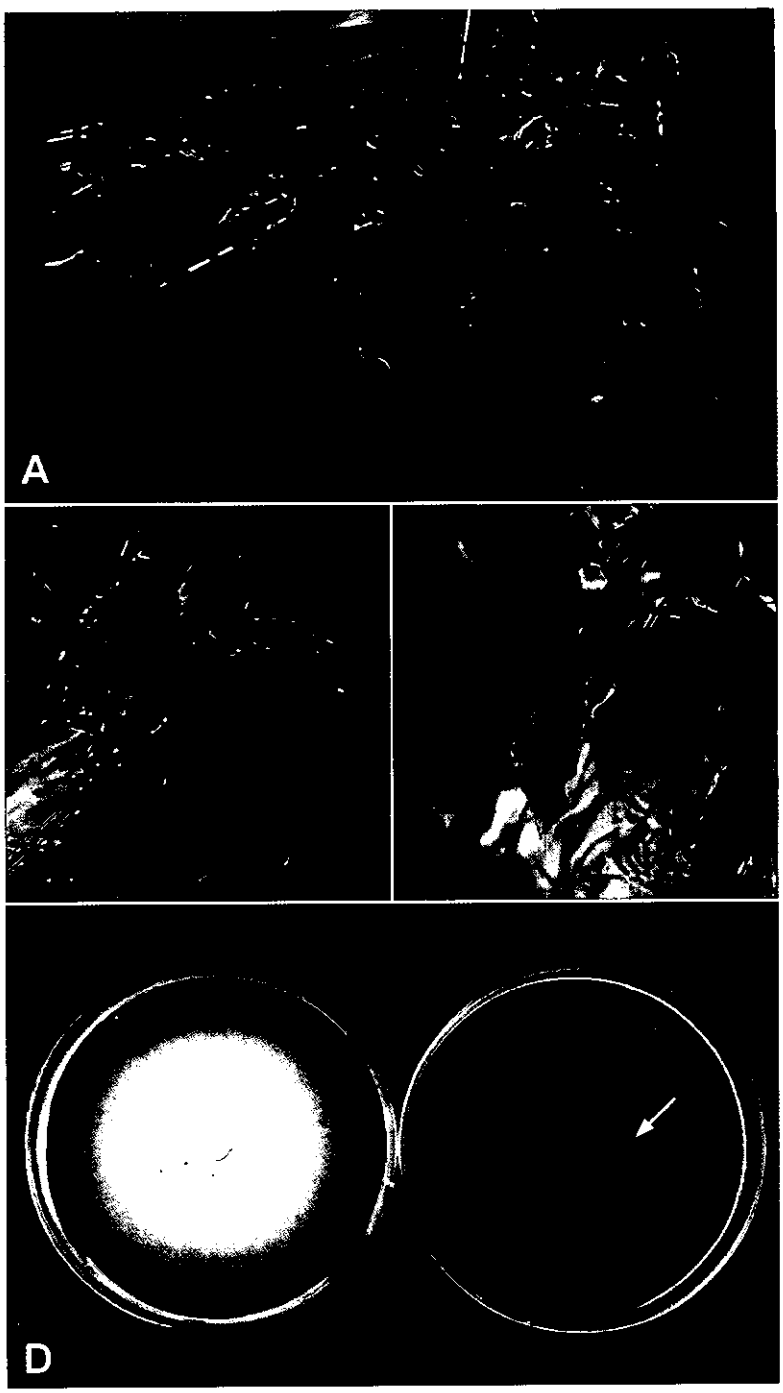

Fig. 1. Symptoms of Verticillium wilt of eggplant, and mycelial colony of *V. dahliae*. (A) Eggplants showing symptoms of Verticillium wilt in a greenhouse. (B) An eggplant showing chlorotic leaves and premature defoliation. (C) Foliar symptoms of Verticillium wilt. Note gradual yellowing, interveinal necrosis, and marginal crinkling of leaves. (D) Mycelial colony of *V. dahliae* grown on PDA. The 2-week-old culture (left) was whitish to creamy, whereas the 4-week-old culture (right) was black due to the formation of numerous microsclerotia (an arrow).

between *V. albo-atrum* and *V. dahliae* has been a subject of controversy since 1913 (Isaac, 1967). It has been generally accepted that *V. dahliae* is distinguished from *V. albo-atrum* by the presence of microsclerotia (Hawksworth and Talboys, 1970a; Hawksworth and Talboys, 1970b). Although *V. tricorpus* also forms microsclerotia, it is distinguished from other species by the formation of chlamydospores, which are absent from *V. dahliae* (Hawksworth, 1970).

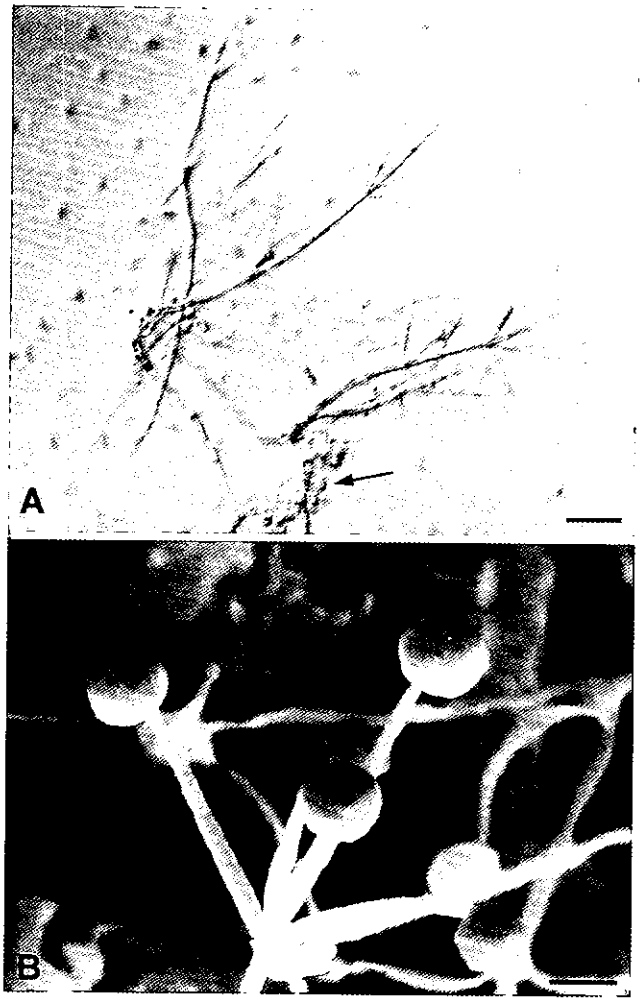

Fig. 2. Mycological characteristics of *V. dahliae*. (A) A differential interference contrast micrograph of verticillately branched conidiophores bearing phialides of *V. dahliae*. Microsclerotia were also found at the base of conidiophores (an arrow). Bar=5 μm. (B) A scanning electron micrograph of phialides of *V. dahliae* grown on PDA. Bar=10 μm.

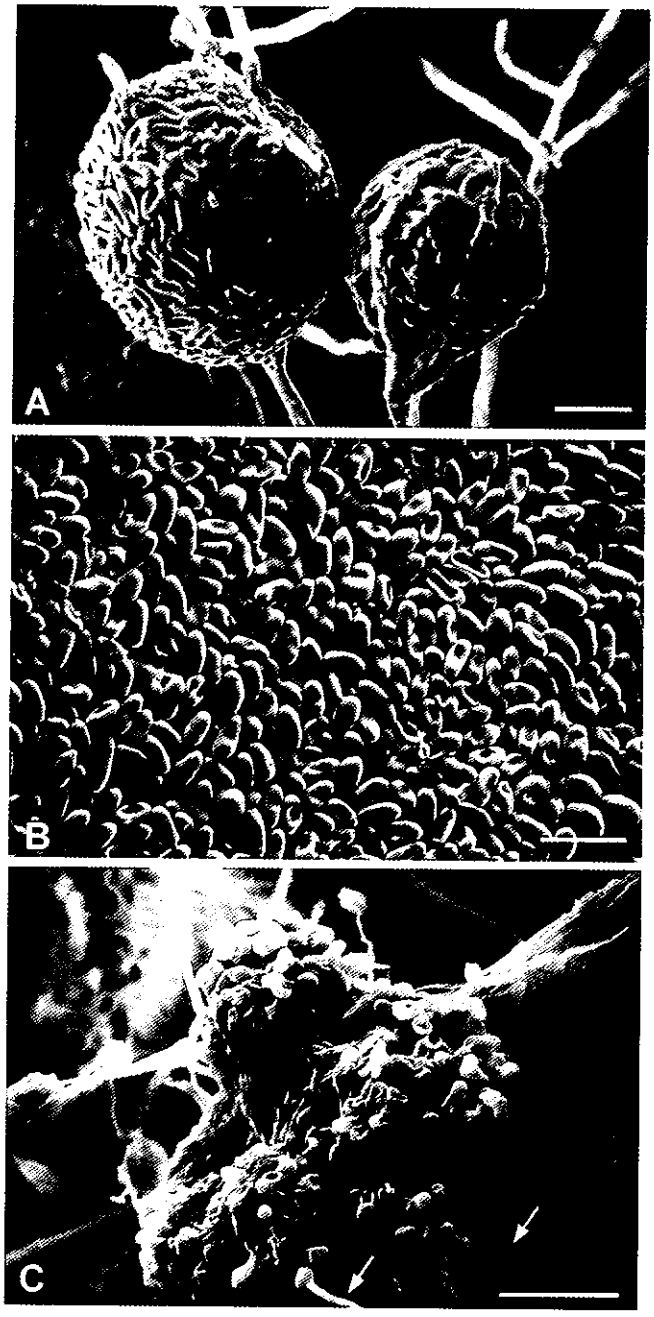

Fig. 3. Scanning electron micrograph of *V. dahliae*. (A) Conidia borne in clusters at the tips of phialides of *V. dahliae*. Bar=10 μm. (B) Conidia placed on the surface of PDA. Bar=10 μm. (C) A microsclerotium consisting of globular cells. Some globular cells produced germ tubes (arrows). Bar=20 μm.

Hawksworth and Talboys (1970b) proposed that many *Verticillium* isolates once identified as *V. albo-atrum* should be referred to as *V. dahliae*.

Inoculation to eggplants induced similar symptoms as observed in naturally infected eggplants, confirming pathogenicity of the isolate. However, differences in disease development were observed with respect to the inoculation methods employed in this study. The inoculum may have been introduced directly into the root zone by soaking roots in the conidial suspension in the RC and RD methods, as proposed in Verticillium wilt of cocoa (Resende et al., 1995). It is considered that such direct contact with roots would be a reason for higher levels of disease incidence and severity than those of seedlings inoculated by the SD method. The lower level of disease development of seedlings inoculated by the SD method may reflect difficulties in fungal invasion and colonization of an intact root system.

Chemical control or crop rotation have been proved not to be effective in reducing losses from Verticillium wilt, because the pathogen is predominantly soil-borne, and can persist as microsclerotia in the absence of host plants for

Table 1. Comparison of morphological characteristics of the isolate (VD-9833) with *V. dahliae* described previously

Characteristics	Isolate (VD-9833)	*V. dahliae* Klebahn[a]
Culture Color	Whitish to creamy Later becoming black	Whitish to creamy Later becoming black
Conidiophores		
Color	Hyaline	Hyaline
Shape	Verticillately branched	Verticillately branched
Phialides		
Color	Hyaline	Hyaline
Size	17.5-32.5 × 2-3 μm	16-35 × 1-2.5 μm
Numbers	3 or 4 at each node	3 or 4 at each node
Conidia		
Color	Hyaline	Hyaline
Size	5-8.8 × 2-4 μm	2.5-8 × 1.4-3.2 μm
Shape	Ellipsoidal to sub-cylindrical	Ellipsoidal to sub-cylindrical
Septa	1-celled or 1-septate	1-celled or 1-septate
Microsclerotia		
Color	Black	Dark brown to black
Size	20-140 × 20-90 μm	Variable
Shape	Globose to irregularly spherical	Elongate to irregularly spherical
Chlamydospores	Absent	Absent

[a]Description by Hawksworth and Talboys (1970b).

many years (Marois et al., 1982). Crop rotation may not be able to reduce a build-up of the inoculum in the soil, because several crops which may be used in rotation with eggplants are also susceptible to *V. dahliae* (Resende et al., 1995). As a result, breeding for resistance may be an alternative for reducing the disease, and some related *Solanum* species have been used as sources of resistance in breeding programs (Alconero et al., 1988). Biological control or cultural practices were also employed to reduce losses from the disease (Elmer and Ferrandino, 1991; Elmer and Ferrandino, 1994; Marois et al., 1982). Effective management strategies and detailed epidemiological studies on the disease occurrence await further investigation.

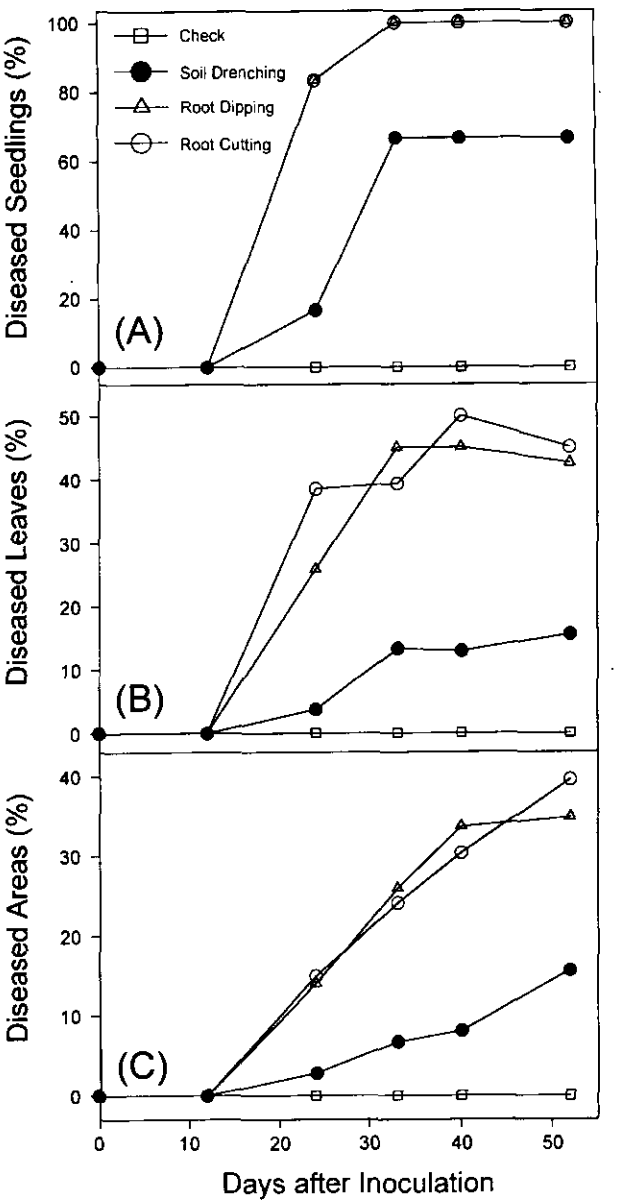

Fig. 4. Temporal progress of Verticillium wilt of eggplant under three inoculation methods. (A) Diseased seedlings. (B) Diseased leaves of eggplants. (C) Diseased areas in eggplant leaves.

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