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Ecopathological Analysis of *Apple stem grooving virus*-K Harboring *Talaromyces flavus*

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Pear black necrotic leaf spot (PBNLS) on pear trees (*Pyrus pyrifolia*) is caused by a Korean isolate of *Apple stem grooving virus* (ASGV-K). Yellow spots were detected in *Phaseolus vulgaris* (kidney bean) and *Chenopodium quinoa* which were grown near the diseased pears in year 2000 through 2003. The ASGV-K, the causative agent of PBNLS, was detected from the symptoms of the diseased kidney bean plant and *C. quinoa*. ASGV-harboring fungi were also isolated from symptomatic plants and from soils surrounding the infected plants. The ASGV-harboring fungus was identified and characterized as *Talaromyces flavus*. Ecopathological studies showed that the number of ASGV-harboring fungi on the pear leaves was not correlated with differences in temperature or severity of symptoms. Additionally, there was no difference in fungus frequency among the orchard locations or different host plants. Although the frequency of fungi isolated from the soil was not affected by changes in temperature or location, the fungi occurred at higher densities in the rhizosphere than in the plants themselves.

Keywords **:** *Apple stem grooving virus*, fungal population, pear black necrotic leaf spot, *Pyrus pyrifolia*, vector transmission

Pear black necrotic leaf spot (PBNLS) was first reported in Korea in 1979, and this severe disease has since spread sporadically through orchards, causing losses of up to 50% in crop yield (Nam and Kim, 1994). PBNLS first appears as small, defined, black spots on mature leaves. The spots, which may have a circular or irregular shape and are approximately 1-4 mm in diameter, are reddish brown during the early stages of the infection and later turn black. These lesions grow larger over time and fuse with neighboring spots until the whole leaf becomes black (Nam and Kim, 1994). PBNLS develops on the basal portion of early emerging leaves and on hardening leaves appearing from leaf buds in late May (Nam and Kim, 1994). The most typical symptom of PBNLS is the appearance of necrotic spotting on mature leaves.

The suggestion by Nam and Kim (2002) that PBNLS was caused by a virus has been investigated experimentally by ultrastructural observations and grafting assays. Shim et al. (2004) reported that the causative agent of PBNLS is a Korean isolate of *Apple stem grooving virus* (ASGV-K), which they detected by electron microscopy and serological analysis, and they reported the full length sequence of the putative pear virus genome as well.

ASGV was identified in diseased *Phaseolus vulgaris* and *Chenopodium quinoa* which had no physical connections to the roots of virus-infected *P. pyrifolia*. Transmission of ASGV from fruit trees to herbaceous plants in nature has not been previously reported, but recently we observed that ASGV could transmit from virus infected pear tree to virus-free pear tree (Shim, 2004). However, the vector mediating this ASGV transmission has not been reported (Lister, 1970).

We hypothesized that ASGV transmission might be vector mediated because ASGV caused epidemics in the field, passing from ASGV-infected pears to virus-free pears, as well as from ASGV-infected pears to herbaceous plants located close to ASGV-infected pears. In this study,

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we isolated an ASGV-harboring fungus from ASGV-infected plants and from soil within the rhizosphere, characterized the fungus, and surveyed the pathoecological distribution of this fungus.

Materials and Methods

Fungal isolates. Samples were recovered from plants showing typical symptoms caused by ASGV from two provinces (Suwon and Anseong) in the Republic of Korea. Leaves, bark, and roots were rinsed with running tap water to remove soil and plant debris. The tissues were then cut using a sterilized scalpel. Small pieces of tissue (1 cm^2) were surface-sterilized in 70% ethanol for 10 s, followed by 1 min in 1% sodium hypochlorite (NaOCl), and were then placed on 2% water agar. Plates were incubated at 28°C and/or 37°C for 2-3 days, after which fungal colonies were transferred to potato dextrose agar (PDA). Ninety mililiter of sterile distilled water was thoroughly mixed with the ten grams of soil and final density of fungus was adjusted to 10^3-10^5/100 μl. This fungus was incubated at 37°C for 1 h. After brief centrifugation, the supernatant was spread onto PDA plates and cultured at 28°C and/or 37°C for 2-3 days. In order to count the frequency of fungus on external and internal leaf tissues, one or two leaves were rinsed with 10 ml of distilled water for the isolation of fungus from external leaf tissues. The frequency of fungi for internal tissues were analyzed by cultures from one or two surface sterilized leaves on PDA plates.

Identification of fungal isolates. The fungal identifications were based on the methods of Samson (1990). The ASGV-harboring fungi were 3-point inoculated on malt extract agar (MEA) and PDA, and incubated in the dark for 7 days at 25°C. After incubation, the diameters of the macroscopic colonies (in millimeters) were measured from the reverse side of the plate, and the range observed on each medium from the three inoculation points was recorded. Colony appearance was judged by eye or with a hand lens or stereomicroscope. To determine colony colors, colonies were examined by daylight or using a daylight-type fluorescent light. To determine whether selected isolates were *Talaromyces flavus*, the ITS-1 and ITS-2 regions were amplified by PCR using gene-specific primers based on the *T. flavus* (U18354) sequence. PCR was also carried out to determine how different the sequences were between different isolates.

RNA extraction. Total RNA was prepared essentially as described by Davis and Ausubel (1989) using the Trizol method (Gibco BRL). Freeze-dried leaves and mycelia were collected in 2 ml tubes, which were quick frozen in liquid nitrogen. The tissue was ground to a fine powder using a mortar cooled in liquid nitrogen. After grinding, the fine powder was added to a 1.5 ml microfuge tube containing 0.55 ml of RNA extraction buffer (0.2 M Tris-HCl, pH 9, 0.4 M LiCl, 25 mM EDTA, and 1% SDS) and 0.55 ml water-saturated phenol. RNA was precipitated by adding a one-third volume of DEPC-treated 8 M LiCl and incubating on ice for 3 h. The RNA pellet was precipitated by centrifugation, dissolved in 0.3 ml DEPC-treated H_2O, and ethanol-precipitated in the presence of 0.3 M sodium acetate, pH 5.2. RNA was collected by centrifugation, dried under vacuum pressure, dissolved in 50 μl DEPC-treated H_2O, and quantified by measuring the absorbance at 260 nm.

RT-PCR. Primers were designed to amplify the coat protein genes of plant viruses known to infect both trees and herbaceous plants (Table 1) (http://www.virusbank.org). The specific primers for *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem pitting virus* (ASPV) were designed based on the recommendations of Menzel et al. (2002).

RT-PCR was carried out using samples of symptomatic plants and isolated fungi. The cDNA synthesis reaction was performed as follows: A single cycle (consisting of 10 min at 70°C, 50 min at 42°C, 5 min at 95°C) was used to reverse transcribe 5 μg of RNA treated with RQ-1 DNase (Promega Madison, WI, USA) in a reaction volume of 30 μl (final concentrations of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5.5 mM $MgCl_2$, 500 μM each dNTP, 0.4 units RNase inhibitor/μl, 1.25 units reverse transcriptase/μl). Samples of the resultant cDNA (5 μl) was then subjected to PCR amplification (5 min at 93°C, followed by 30 cycles of 20 s at 94°C, 40 s at 57°C, 40 s at 72°C) using Hi-Fidelity PCR amplification reagents (Super-bio). For coat protein (CP) gene amplification, the primer pairs used were: ASGV-P1, 5'-CAT ATG AGT TTG GAA GAC GTG CTT C-3'; and ASGV-P2, 5'-CTC GAG ACC CTC CAG TTC CAA GTT A-3'. The PCR product was sub-cloned into the pGEM-T Easy vector (Promega), according to the manufacturer's instructions.

Results and Discussion

Symptoms of ASGV were observed in *C. quinoa* and *P. vulgaris* plants which were grown within around 160 m^2 of pear orchard that exhibited signs of PBNLS. But no symptoms were observed on *C. quinoa* and *P. vulgaris* or other plants cultivated in fields more distantly located from pear trees exhibiting PBNLS (Fig. 1).

RT-PCR was performed to determine whether a virus was causing the disease. The PCR primers for the amplification

Table 1. Primers used for amplification and detection of various plant viruses

Viruses	Primer sequences (5'-3')	Size (bp)	RT-PCR
ACLSV (Apple chlorotic leaf spot virus)	atggcagcagttctgaatttgcagc ctaaatgcaaagatcagtcgacaca	581	–
ASPV (Apple stem pitting virus)	atggcttccgacggatcccagcctc tgtagacttgtctgaggcgccaaca	720	–
ASGV (Apple stem grooving virus)	atgagtttggaagacgtgcttcaa ctaaccctccagttccaagttact	699	+
BGMV (Bean golden mosaic virus)	atgcctaagcgtgatgctcaatg ttaattggtgatcgaatcataaa	755	–
BYMV (Bean yellow mosaic virus)	caatgcaggtggggtgaaggat cgtggcatatacctctctgttg	552	–
CarMV (Carnation mottle virus)	atggaaaataaaggagaaaa agcagcgagagttgagggaa	368	–
CMV (Cucumber mosaic virus)	atggacaaatctgaatcaac tcagactgggagcaccccag	657	–
CPMV (Cowpea mosaic virus)	tcaggaactgggcagccgcaac ctgcggggaacccatgccaaga	860	–
LSV (Lily symptomless virus)	atgcaatcaagaccagctca aggttgatttctcacgagca	600	–
PLRV (Potato leaf roll virus)	atgagtacggtcgtggttag tgcaaagccaccctgatggt	614	–
PVY (Potato virus Y)	atggtttggtgcattaaaaa catcctcggtggtgtgcctc	419	–
SMV (Soybean mosaic virus)	ccaggcaaggagaaggaaggag ttactgcggtgggcccatgccc	795	–
TMV (Tobacco mosaic virus)	agttaattaatttatgtact atttaagtggagggaaaaac	503	–
ToMV (Tomato mosaic virus)	ctttccctcagagcaccgtc caacccagacatactttcaa	290	–
WMV (Watermelon mosaic virus)	atggtttggtgtatcgagaa gctgcggccttcatttgtgc	326	–
ZyMV (Zucchini yellow mosaic virus)	tggtgtatagaaaacggcac ctgcattgtattcacaccta	453	–

of virus coat proteins were designed by sequence analysis of viruses reported in the Republic of Korea (Choi, 1999; Ryu, 2002) (Table 1). The PCR conditions and specificity were determined using viruses supplied by the Rural Development Administration (RDA) and the Plant Virus GenBank.

RT-PCR was performed with total RNA extracted from the symptomatic leaves of pears, kidney beans, and *C. quinoa*. The ASGV CP gene was amplified from all tissues (Fig. 1D), and sequence analysis demonstrated that the sequence was identical to the ASGV CP gene sequence in GenBank (Fig. 1E). Additionally, the CP genes of bean golden mosaic virus (BGMV), bean yellow mosaic virus (BYMV) and soybean mosaic virus (SMV) were not amplified from kidney bean plants that showed virus-like symptoms. No product was detected from any of the other PCR primer sets shown in Table 1 (data not shown).

It is possible that the causative agent of the disease might be associated with a soil microorganism because: (1) viral disease symptoms occurring from year 2000 to 2003 were observed in kidney beans located near ASGV-infected pears, but peppers, sesames, and cabbages did not show any symptoms; (2) new kidney beans grown in furrows developed the same disease symptoms detected in kidney

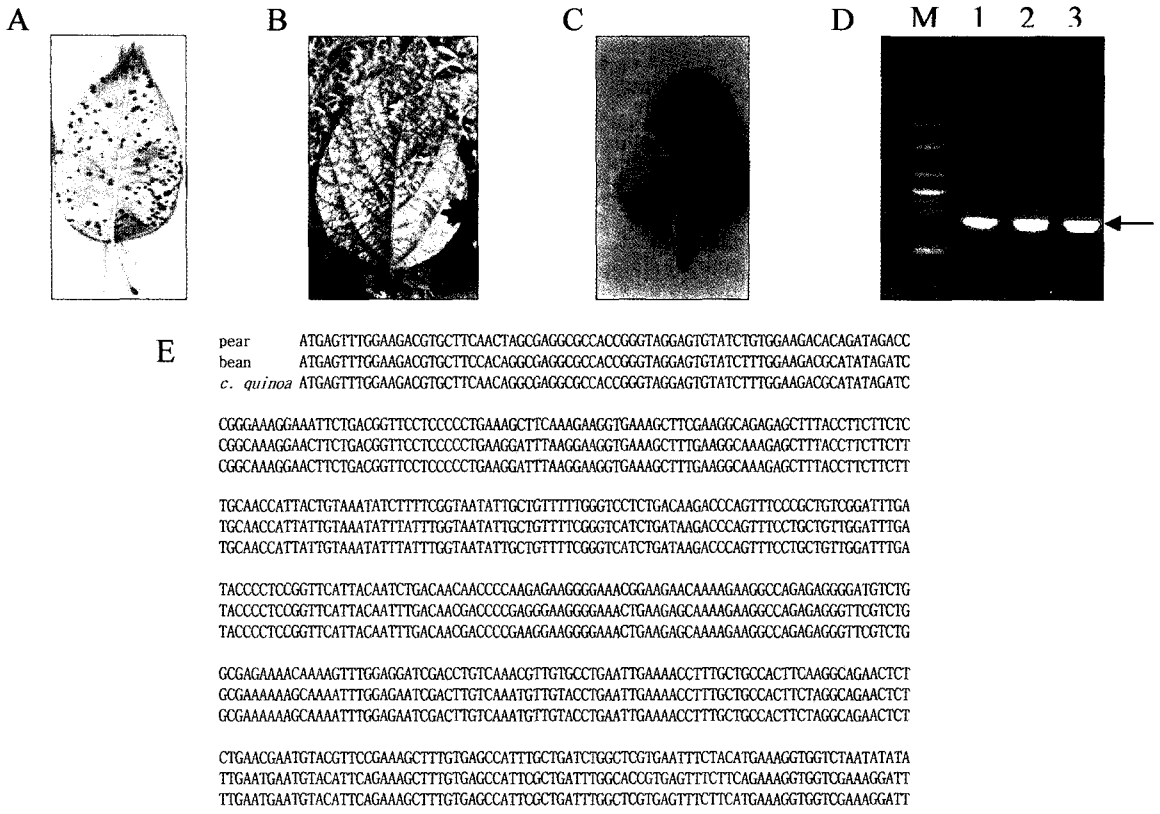

Fig. 1. Typical pear black necrotic leaf spot disease symptoms as they appear in *Pyrus pyrifolia* (A), *Phaseolus vulgaris* (B), and *Chenopodium quinoa* (C). Leaves were taken from plants infected in the field. (D) RT-PCR and sequence analysis of ASGV CP gene from plants showing ASGV-like symptoms. RT-PCR was performed with specific primers (ASGV-P1 and ASGV-P2) for the ASGV CP gene. M, 100 bp DNA molecular weight marker (Bioneer); Lane 1, *P. pyrifolia*; Lane 2, *P. vulgaris*; Lane 3, *C. quinoa*. (E) Nucleotide sequence alignment of amplified products and the previously reported ASGV CP gene sequence (GenBank AF465354). Sequence analysis was carried out with the program provided at http://www.toulouse.inra.fr/multalin.html.

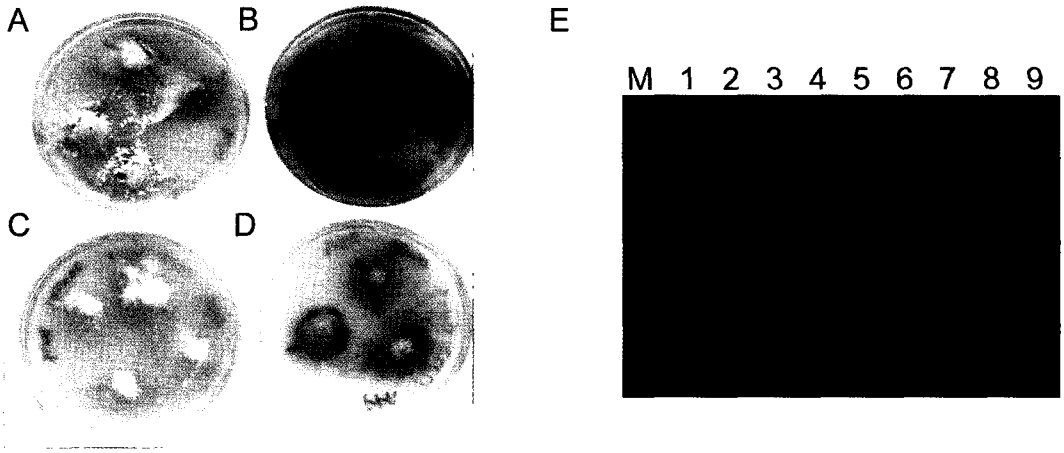

Fig. 2. Morphology of fungi isolated from soil or virus-infected plant tissues. (A) fungi isolated from roots of a virus-infected pear (B) fungi isolated from rhizosphere soil of a virus-infected pear (C) fungi isolated from roots of a virus-infected kidney bean (D) fungi isolated from rhizosphere soil of a virus-infected kidney bean. (E) RT-PCR for virus detection of in samples of fungi isolated from pear and kidney bean plants and surrounding soils. M, 100 bp DNA size marker; Lane 1, fungi cultured from diseased pear root; Lane 2, fungi cultured from soil (pear); Lane 3, fungi cultured from diseased bean root; Lane 4, fungi cultured from soil (bean); Lane 5, ACLSV; Lane 6, ASPV; Lane 7, BGMV; Lane 8, BYMV; Lane 9, SMV. The ASGV CP-specific primers were used in lanes 1-4.

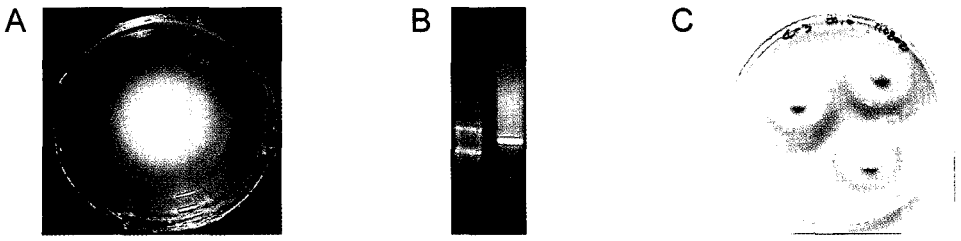

Fig. 3. Virus-infected strain isolation. (A) Culture morphology and mycological characteristics of ASGV-harboring fungi. This strain showed dense mycelia and pigmentation ranging from yellow to yellowish-green in the center and white at the margins. (B) ASGV CP gene was amplified from this strain by RT-PCR. (C) Culture morphology and mycological characteristics of ASGV-infected *Talaromyces flavus*. *T. flavus* grew rapidly on MEA, with dense mycelia and pigmentation ranging from yellow to yellowish-green in the center and white at the margins.

beans during the previous year; and (3) the soil of the orchard was not exchanged or covered with soils from other areas. We were indeed able to isolate and identify the soil microorganism that transmitted and carried the plant virus.

After CP genes were identified from the leaves of *P. pyrifolia* and *P. vulgaris*, fungi were cultured from the infected plants and the soil around the plants. Among soil-borne microorganisms, fungi and nematodes are known to transmit viruses. Nematodes were not isolated from the tissues or soils, but many fungi were isolated from different plant tissues and from the soil (Fig. 2).

Total RNA was extracted from fungi isolated from soils and root tissues, and RT-PCR was then performed using ASGV CP-specific primers. RT-PCR for three bean viruses (BGMV, BYMV, and SMV) and two pear viruses (ACLSV and ASPV) was also performed, but only the ASGV CP gene was amplified by RT-PCR, which was confirmed by sequence analysis (Fig. 2E).

Single conidia were isolated from fungi pools with ASGV and grown on PDA plates for 14 days at 28 °C. Total RNA was extracted for RT-PCR with ASGV CP-specific primers. The ASGV CP gene product was amplified from one of the PDA plates (Fig. 3B). After culturing for seven days, a pale green circle had formed around the tips of the mycelia (Fig. 3A). Another RT-PCR amplification using ASGV CP-specific primers was performed to confirm that the fungus contained ASGV.

Two orchards, one near Suwon and one near Anseong, were used to study the distribution, frequency, and ecology of the fungus. Two experimental sites were selected for each orchard. Kidney bean was used as the indicator plant at one site, and sesame was used at the other site. To analyze the pathoecological distribution of fungi infected with ASGV, diseased plants with typical symptoms and rhizosphere soil were used for isolation of fungi (Table 2). Control fungus was prepared and isolated from asympto-

Table 2. Isolation of suspected fungal vectors of ASGV from the rhizoplane of pear and kidney bean growing under and near pear trees exhibiting PBNLS

(A) List of soil samples

Sample no.	Host	Condition	Collection area
R-1	Pear	ASGV-infected plants	Suwon (NIAB[1])
R-2	Bean	Virus-like symptoms	Suwon (NIAB)
R-3	Bean	Virus-like symptoms	Suwon (NIAST[2])
R-4	Pear	Healthy plants (no visible symptoms)	Suwon (NIAST)

[1]National Institute of Agricultural Biotechnology
[2]National Institute of Agricultural Science and Technology

(B) Fungal population densities in the rhizoplane of pear and kidney bean growing under and near pear trees exhibiting PBNLS

Sample no.	No. of cfu/g root ($\times 10^3$)			Average ($\times 10^3$)	No. of isolates selected for further testing
	I	II	III		
R-1	0.83	1.49	0.99	1.1	9
R-2	8.33	13.33	6.66	9.4	7
R-3	6.66	14.99	6.66	9.4	9
R-4	2.02	1.06	1.49	1.52	10

Table 3. Isolation of suspected fungal vectors from rhizosphere soils of pear trees exhibiting PBNLS

(A) List of soil samples

Sample no.	Host	Condition	Collection area
S-1	Pear	ASGV-infected plants	Suwon (NIAST[1])
S-2	Pear	ASGV-infected plants	Suwon (NIAB[2])
S-3	Pear	Healthy plants (no visible symptoms)	Suwon (NIAB)
S-4	Sesame	Negative control for this study	Suwon (NIAST)

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[2]National Institute of Agricultural Biotechnology

(B) Fungal population densities in the rhizosphere of pear trees exhibiting PBNLS

Sample no.	No. of cfu/g root ($\times 10^3$)			Average ($\times 10^3$)	No. of isolates selected for further testing
	1st Exp	2nd Exp	3rd Exp		
S-1	1.75	2.25	0.50	1.50	8
S-2	3.75	4.25	3.25	3.75	9
S-3	1.00	1.50	1.17	1.17	10
S-4	7.25	6.00	7.00	6.75	7

matic pear leaves, sesame leaves, and soils surrounding these plants (Table 3). A total of 69 fungi were selected for further analysis. Three ASGV positives were sent to independent laboratories [Centraalbureau voor Schimmelcultures (CBS) in the Netherlands and Korean agricultural culture collections (KACC) in the Republic of Korea] for identification of each isolate.

A selected ASGV carrying fungus grew well on PDA plate and formed ascomata (Fig. 3C). This isolate formed

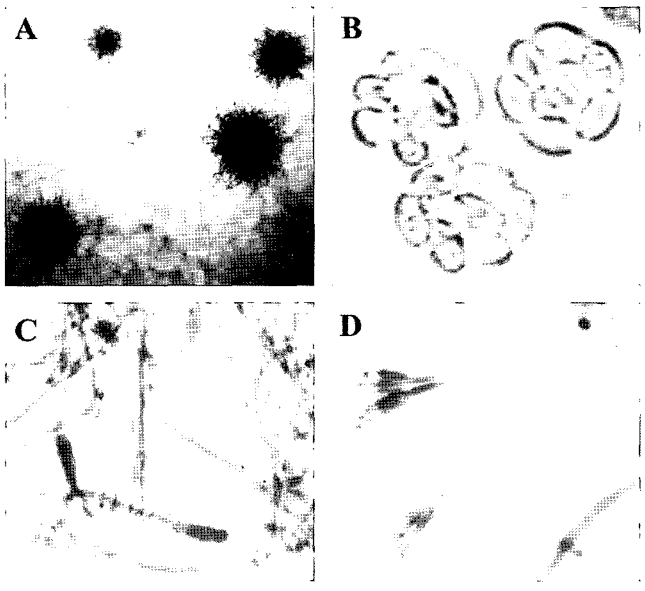

Fig. 4. Characteristic features of *T. flavus*. The conidiophore was branched in a brush-like manner, with phialides at the branch tips. (A) ascomata (B) ascospore (C) antheridia and ascogonia (D) biverticillate *Penicillium* sp.

many circular asci bearing eight spiny ascospores (Fig. 4B). The sizes of the asci and ascospores were 7-9 x 7-9 μm and 4.4-5.6 x 2.4-3.6 μm, respectively, and these characteristics were similar to the original description of *T. flavus*. Lobe-shaped ascogonia and antheridia surrounding the ascogonia showed typical characteristics of *T. flavus*, and these structures were observed in the fungus described here. This fungus isolate did not grow at 4°C, but grew actively at temperatures from 25 to 37°C. The anamorphic name of this fungus was biverticillate *Penicillium* sp. (Fig. 4D). Also, ITS-1 and ITS-2 regions of ASGV-harboring fungus were amplified and compared with sequences of *T. flavus* (U18354). The sequences of *T. flavus* (U18354) and ASGV-harboring fungus were identical each other (data not shown). Our results were verified by two independent laboratories, CBS and KACC, confirming that the fungus isolated from pear and kidney bean was *T. flavus*.

The frequency and distribution of fungi were surveyed from different developmental stages of *Pyrus* trees and under different temperature conditions. Fungi were isolated from flowers, internal and external tissues of leaves of ASGV-infected pears, and healthy pears, as well as from soil around the pear plants. ASGV-harboring fungi were identified in the surveyed fungal isolates by RT-PCR analysis. The changes in frequency and distribution of ASGV-harboring fungi were analyzed by comparison to the total population of fungi surveyed in this study. The survey was conducted for three months in different orchards because pear plants infected with ASGV first show symptoms during this season. Information regarding the frequency and distribution of fungi came from total numbers of fungi isolated from soils and pears, not from

individual plants. from pears flower for three months. A total of 89 and 16 isolates were isolated from the two separate orchards, respectively, during this period, but ASGV-harboring fungi were not detected (data not shown).

The frequency of fungi isolated from leaf surfaces decreased by 50% for three months. The number of ASGV-harboring fungi was not affected by temperature changes or severity of symptoms, and there were no differences between the two locations (data not shown).

No ASGV-harboring fungi were isolated from the interior of the leaves during the month of April, although many fungi were isolated from leaf surfaces during this period. Relatively more fungi were isolated from the interior of the leaves in May, and the numbers then decreased in June. Additionally, no difference in fungal frequency was observed among the different orchard locations and host plants.

Data regarding soil was quite different from those of leaves and flowers. Relatively constant numbers of fungi were isolated from the soil (data not shown). The frequency of fungi isolated from the soil was not affected by changes in temperature. The average frequency of fungal isolation was 13 isolates per gram of soil. The frequency of ASGV-harboring fungi was similar to the pattern of total fungal frequency. Soil contained relatively more ASGV-harboring fungi than did flowers and leaves.

Acknowledgements

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References

Adams, M. J. 1991. Transmission of plant viruses by fungi. *Ann. Appl. Biol.* 118:479-492.

Ahmed, N. A., Christie, S. R. and Zettler, F. W. 1983. Identification and partial characterization of a closterovirus infecting *Nandina domestica*. *Phytopathology* 73:470-475.

Caston, J. R., Ghabrial, S. A., Jiang, D., Rivas, G., Alfonso, C., Roca, R., Luque, D. and Carrascosa, J. L. 2003. Three-dimensional structure of *Penicillium chrysogenum* virus: A double-stranded RNA virus with a genuine T=1 capsid. *J. Mol. Biol.* 331:417-431.

Choi, J. K. 1999. http://bric.postech.ac.kr/biowave/review

Chu, Y. M., Jeon, J. J., Yea, S. J., Kim, Y. H., Yun, S. H., Lee, Y. W. and Kim, K. H. 2002. Double-stranded RNA mycovirus from *Fusarium graminearum*. *Appl. Environ. Microbiol.* 68:2529-2534.

Chu, Y. M., Lim, W. S., Yea, S. J., Cho, J. D., Lee, Y. W. and Kim, K. H. 2004. Complexity of dsRNA mycovirus isolated from *Fusarium graminearum*. *Virus Genes* 28:135-143.

Davis, K. R. and Ausubel, F. M. 1989. Characterization of elicitor induced defense responses in suspension-cultured cells of Arabidopsis. *Mol. Plant-Microbe Interac.* 2:363-368.

Lister, R. M. 1970. *Apple stem grooving virus.* CMI/AAB Descriptions of plant viruses No. 31.

Menzel, W., Jelkmann, W. and Maiss, E. 2002. Detection of four apple viruses by multiplex RT-PCR assays with co-amplification of plant mRNA as internal control. *J. Virol. Methods* 99:81-92.

Nam, K. W. and Kim, C. H. 1994. Studies on the pear abnormal leaf spot disease. 1. Occurrence and damage. *Korean J. Plant Pathol.* 10:169-174.

Nam, K. W. and Kim, C. H. 2002. Graft transmission and cytopathology of pear black necrotic leaf spot (PBNLS) disease. *Plant Pathol. J.* 18:301-307.

Ryu, K. H. 2002. http://www.virusbank.org

Samson, R. A. and Pitt, J. I. 1990. Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York.

Shim, H. K. 2004. Molecular characteristics and pathological interactions of *Talaromyces flavus* and *Apple stem grooving virus-M.* Ph.D. Dissertation of Sungkyungkwan University, Korea.

Shim, H. K., Min, Y. J., Hong, S. Y., Kwon, M. S., Kim, D. H., Kim, H. R., Choi, Y. M., Lee, S. C. and Yang, J. M. 2004. Nucleotide Sequences of Korean Isolate of Apple Stem Grooving Virus Associated with Black Necrotic Leaf Spot Disease on Pear (*Pyrus pyrifolia*). *Mol. Cells* 18:192-199.

von Wechmar, M. B. 1980. Transmission of brome mosaic virus by *Puccinia graminis tritici*. *Phytopathology* 5:239-250.

von Wechmar, M. B., Chauhan, R. and Knox, E. 1992. Fungal transmission of a potyvirus: uredospores of *Puccinia sorghi* transmit *Maize dwarf mosaic virus*. *Arch. Virol. Suppl.* 5:239-250.