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e-**X**tra*

Pathogenic Specialization of *Venturia nashicola*, Causal Agent of Asian Pear Scab, and Resistance of Pear Cultivars Kinchaku and Xiangli

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ABSTRACT

Scab caused by *Venturia nashicola* is one of the most serious diseases of Asian pears, including Japanese pear (*Pyrus pyrifolia* var. *culta*) and Chinese pears (*P. bretschneideri* and *P. ussuriensis*). Breeding scab-resistant pear cultivars is essential to minimize fungicide use and development of fungicide resistance. A survey of pathogenic specialization in *V. nashicola* is needed to ensure durable scab resistance in cultivated pears. *V. nashicola* race 1, 2, and 3 isolates, each differing in pathogenicity to Japanese pear cultivar Kousui and Asian pear strain Mamenashi 12, have been reported in Japan. In this study, isolates collected from scabbed pears in China and Taiwan were classified as *V. nashicola* based on conidial size and mating ability. However, various isolates had pathogenicity distinct from races 1, 2, and 3 according to tests on seven differential host genotypes of pear cultivars from Japan (Kousui and strain Mamenashi 12), China (Jingbaili, Yali, Linyuli, and Nanguoli), and Taiwan (Hengshanli). These new races were designated as races 4 to 7. Progenies characteristic of race 3 isolates were produced using a cross between race 1 and race 2 isolates, suggesting the possible role of sexual recombination in the emergence of novel races. Japanese pear cultivar Kinchaku and Chinese *P. sinkiangensis* 'Xiangli' (a Korla fragrant pear grown in China) did not show visible symptoms after inoculation with any of the seven races. Broad scab resistance in Kinchaku and Xiangli makes them a promising genetic resource for resistance breeding programs.

Keywords: host–parasite interactions, mating, pathogenicity, pear, race, resistance, scab, sexual recombination, *Venturia*, *Venturia nashicola*

Among deciduous fruit trees, production of pear (*Pyrus* spp.) is second to that of apple worldwide (Saito 2016). The origin and domestication centers of the genus *Pyrus* span China, Central Asia, the Middle East, and Asia Minor (Vavilov 1992). Asian pears, including Japanese pear (*P. pyrifolia* var. *culta*) and Chinese pears (*P. bretschneideri*, *P. ussuriensis*, and others), are grown widely in Japan, Korea, and China. In addition, Asian pears are regarded as a potential alternative fruit crop for growers in the U.S. Mid-Atlantic Region (Walsh et al. 2016).

Two fungal species cause scab disease on pear (González-Domínguez et al. 2017). *Venturia nashicola* causes scab on Asian pears, whereas *V. pyrina* (syn. *V. pirina*) (Rossman et al. 2018) causes scab on European pear (*P. communis*). These pathogens have differing morphological, cultural, and pathological characteristics (Tanaka and Yamamoto 1964). Results from in vitro mating experiments demonstrated that the two species are sexually isolated. *V. nashicola* is pathogenic only on Japanese and Chinese pears, and *V. pyrina* is pathogenic only on European pear (Ishii and Yanase 2000). Additionally, isozyme patterns for esterase and peroxidase are unique to either *V. nashicola* or *V. pyrina* isolates (Ishii and Suzaki 1994), and sequence variations in the ITS1-5.8S-ITS2 internal transcribed spacer region of rDNA indicated that the two species were closely related but genetically distinct from *Venturia* species infecting other tree fruit hosts (Schnabel et al. 1999). Subsequent phylogenetic studies using rDNA-ITS, β-tubulin, elongation factor 1α, and endopolygalacturonase genes support taxonomic separation of *V. nashicola* from *V. pyrina* (Zhao et al. 2012, 2016), as do comparative genomics studies (Prokchorchik et al. 2020).

Scab caused by *V. nashicola* is one of the most serious diseases of commercially grown Japanese and Chinese pears in East Asia but is not known to occur outside of Asia. Consequently, *V. nashicola* is a quarantined pathogen in many countries (Australian Quarantine and Inspection Service 1998; Jeger et al. 2017; Le Cam et al. 2001). To control scab, pear growers rely on frequent fungicide applications because very few scab-resistant cultivars are commercially acceptable. As a result, *V. nashicola* has developed resistance to benzimidazoles and sterol demethylation inhibitor fungicides (Ishii 2012). Therefore, scab-resistant cultivars are the best strategy for sustainable pear production with low environmental impact.

Pear cultivars Kinchaku from Japan and Hongli and Mili from China are highly resistant to scab caused by *V. nashicola* (Abe and Kotobuki 1998; Abe and Kurihara 1993; Ishii et al. 1992). Yutaka (from Hongli × Housui [Hosui], which is scab susceptible) has high fruit quality and is the first commercial pear cultivar with resistance to multiple diseases, including scab, black spot (*Alternaria alternata* Japanese pear pathotype), and anthracnose (*Colletotrichum gloeosporioides* sensu lato) (Ishii and Kimura 2018).

Progenies derived from crosses between Kinchaku or Hongli and a *V. nashicola*-susceptible Japanese pear cultivar segregated in a 1:1 (resistant/susceptible) ratio supporting the hypothesis that a single dominant gene (*Vn*) controls the resistance phenotype (Abe and Kotobuki 1998). Scab resistance gene *Vnk* was subsequently identified in Kinchaku and mapped to linkage group 1 (Terakami et al. 2006). Gonai et al. (2009) concluded that a major gene (*Vnk*) and minor genes control scab resistance in Kinchaku. Similarly, European pear possesses a single dominant gene pair (*Vn*/*Vn*) that controls resistance to *V. nashicola* (Abe et al. 2000). However, for Yutaka, scab resistance was inherited in a more complex manner because progenies from the Yutaka × susceptible cultivar Natsushizuku cross did not segregate in a 1:1 ratio (Ishii and Kimura 2018).

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*The ***e*-X**tra logo stands for "electronic extra" and indicates there are supplementary materials published online.

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Resistance durability is critical when pathogenic specialization can result in the loss of resistance. In the apple/scab pathosystem, almost 20 races of *V. inaequalis* have been defined based on avirulence (*Avr*) loci (Bus et al. 2011; Patocchi et al. 2020). The dominant resistance gene (*Vf* = *Rvi6*), which is most frequently used in resistance breeding, was rendered ineffective with the appearance of a new *V. inaequalis* race (Guérin and Le Cam 2004; Papp et al. 2020; Parisi et al. 1993). Similarly, the formerly saprotrophic fungus *V. asperata* developed the ability to infect apple cultivars carrying the *Vf* resistance gene (Caffier et al. 2012; Turan et al. 2019). Virulent isolates of *V. inaequalis* exist for most scab resistance genes used in apple breeding, except for *Rvi11* and *Rvi15* (Patocchi et al. 2020). Similar information is lacking for the pear/*Venturia* pathosystem; however, Shabi et al. (1973) reported the existence of five *V. pyrina* races using isolates of the fungus from Israel.

To date, only three *V. nashicola* races have been characterized in Japan. Race 1 is pathogenic to the most popular and widely cultivated Japanese pear cultivar Kousui (Kosui) but not to large-fruited Asian pear strain Mamenashi 12 (possibly a hybrid between Chinese sand pear and wild Asian pea pear). Race 2 is pathogenic to Mamenashi 12 but not to Kousui. Finally, race 3 is pathogenic to Kousui and Mamenashi 12 (Ishii et al. 2002). Race 1 isolates are widespread, but those of minor races 2 and 3 are restricted to the west of Japan. The distribution and race status of *V. nashicola* on pear in Japan and elsewhere in the East Asia region is poorly characterized. This study aimed to collect isolates from China and Taiwan, characterize their morphology and mating compatibility in culture, and determine their pathogenicity on pears to establish the scab pathogen species and race distribution in East Asia.

MATERIALS AND METHODS

Sample collection and fungus isolation. Sixty-three isolates were included in this study (Table 1). Leaves or fruit of Chinese pears naturally infected with scab disease were collected from various commercial orchards separated distantly throughout China and sent to the authors in Japan. Single spores were isolated as described in the Supplementary Material. There were 51 isolates from China. One isolate from Taiwan was provided by Wen-Hsin Chung (National Chung Hsing University, Taiwan). This study also included laboratory stock isolates of *V. nashicola* ($n = 9$) and *V. pyrina* ($n = 2$) derived from single spores and stored at the National Institute for Agro-Environmental Sciences in Tsukuba, Japan. Three *V. pyrina* isolates from samples of scabbed leaves of European pear were included as a reference. Imported samples were handled according to the Plant Protection Act under the supervision of the Japanese Ministry of Agriculture, Forestry, and Fisheries. Representative isolates with distinct pathogenicity on differential hosts were deposited at the National Agriculture and Food Research Organization Genetic Resources Center in Tsukuba, Ibaraki, Japan (Table 1).

Conidial measurements. Conidia were collected directly using a paint brush and distilled water (DW) from leaves or fruit of naturally infected Chinese pears in Liaoning, Shaanxi, Shandong, Anhui, Yunnan, and Jilin provinces in China. For the Taiwanese isolates, conidia were obtained from leaves of inoculated Taiwanese pear cultivar Hengshanli as described earlier. The collected conidia were suspended in DW, and a drop of the suspension was placed on a glass slide. A coverslip was placed over the droplet, and 50 conidia chosen arbitrarily from these samples were measured using a light microscope at ×400 and a Nikon Digital Sight measuring apparatus (Nikon, Tokyo, Japan). Conidial sizes were compared statistically based on 95% confidential intervals (95% CIs).

Mating experiments. *V. nashicola* is a heterothallic fungus. Parental monoconidial isolates were crossed in culture, and the formation and gemination of ascospores were inspected microscopically (details are provided in the Supplementary Material).

Pathogenicity tests. *Preliminary experiments.* Conidial suspensions prepared from Kousui leaves sampled in Japan were sprayed on young leaves of potted Kousui and Chinese pear cultivar Jingbaili trees. Conidial suspensions were also prepared from scabbed leaves of Jingbaili grown in Liaoning, China, but this conidial suspension was drop inoculated onto the main vein of young leaves of potted Jingbaili, Kousui, Kinchaku, Mamenashi 12, and Flemish Beauty trees.

Inoculation with conidia produced in cellophane culture. For inoculum, conidia of monoconidial isolates were produced in culture using the methods described in the Supplementary Material. However, the cultures produced very few conidia, and some isolates did not produce sufficient conidia to repeat the inoculation experiments despite repeated culturing attempts. As a result of inconsistent conidia production in culture for isolate Jilin 1-4, conidia that formed on leaf lesions of inoculated potted trees of Chinese pear cultivar Nanguoli (the original host) were substituted for conidia from cultures and used. Conidia, regardless of source, were suspended in 0.01% vol/vol Tween 80 and 0.1% wt/vol sucrose in DW. Conidia from culture were adjusted to approximately 1.0×10^5 conidia ml^{-1} and suspensions of conidia from lesions were adjusted to 2.5 to 5.0×10^5 conidia ml^{-1} (Ishii and Yanase 2000). Suspensions were stored in a freezer at –30 or –80°C until use.

Pathogenicity tests were conducted between 2001 and 2014 using pear cultivars from Japan (Kousui and Kinchaku), China (Jingbaili [Beijingbaili], Yali, Linyuli, and Nanguoli), and Taiwan (Hengshanli), along with *P. sinkiangensis* 'Xiangli' (fragrant pear, Korla Xiangli), Asian pear strain Mamenashi 12, and nonhost European pear cultivar Flemish Beauty. Soon after leaf emergence, young leaves of 2- to 5-year-old potted trees prepared from the same clone, cut-grafted to seedling stock of wild pear, and grown outside with standard management were either drop inoculated with cultured conidia suspension or sprayed with conidia collected from trees using a plastic hand sprayer. For drop inoculations, 5 to 20 µl of suspension was dropped onto three sites on the main vein of three to five leaves on the same tree and allowed to dry. For spray inoculations, both sides of young leaves were sprayed until run-off. Inoculated and uninoculated plants (used as a control) were incubated at 20°C in a high-humidity chamber with 95 to 100% relative humidity (Koitotron TH; Koito Electric Industries, Ltd., Shizuoka, Japan) for 48 h in the dark and subsequently at 25°C in a phytotron under natural light until scab development was assessed 1 month after inoculation. Pathogenicity of individual isolates was judged visually based on the presence or absence of conidial formation on drop-inoculated or spray-inoculated leaves. The percentage of scab incidence was calculated as follows: (number of sporulating sites/number of drop-inoculated sites) × 100. Inoculation experiments were repeated independently at least one or more times as long as inoculum was available.

Potential role of sexual reproduction in the emergence of new races. Representative race 1 and race 2 isolates (Yasato 2-1-1 and Mamenashi 12A no. 1-4, respectively) were crossed to produce ascospores (details are provided in the Supplementary Material). Thirty-six monoascosporic isolates were cultured on cellophane until conidia formed. Leaves of potted Kousui and Mamenashi 12 trees were drop inoculated, incubated, and assessed for the presence or absence of conidial formation on inoculated sites as described previously.

RESULTS

Conidial size. The mean conidia size (length × width) from Chinese and Taiwanese pears was 15.9 × 7.8 µm (range, 6.4 to 27.9 µm long and 3.7 to 14.7 µm wide). This size was significantly shorter than that of *V. pyrina* from European pear cultivars William and Flemish Beauty (20.4 × 7.6 µm; range, 11.9 to 31.2 µm long and 3.1 to 11.8 µm wide) used as a reference (Table 2). 95% CIs for mean length and width were ±0.58 and ±0.29 µm for conidia

TABLE 1. Collection information and other metadata for *Venturia nashicola* and *V. pyrina* isolates from pear used in this study

Species	Race[a]	Isolate	Host	Cultivar or strain	Location (collection year)	MAFF no.[b]	Reference[c]
V. nashicola	1	JS-115	Japanese pear	Hakko	Oita, Japan (1980)	615015	Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	1	Yasato 2-1-1	Japanese pear	Kousui or Housui	Ibaraki, Japan (1992)	306981	Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	1	OYO-1	Japanese pear	Unknown	Tochigi, Japan (1999)	306982	Zhao et al. (2012)
V. nashicola	2	Mamenashi 12A no. 1-1	Asian pear strain	Mamenashi 12	Tottori, Japan (1991)	615016	Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	2	Mamenashi 12A no. 1-3	Asian pear strain	Mamenashi 12	Tottori, Japan (1991)		Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	2	Mamenashi 12A no. 1-4	Asian pear strain	Mamenashi 12	Tottori, Japan (1991)	615031	Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	3	Mamenashi 12B no. 1-2	Asian pear strain	Mamenashi 12	Tottori, Japan (1991)	306983	Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	3	Mamenashi 12B no. 53-1	Asian pear strain	Mamenashi 12	Tottori, Japan (1992)	615032	Ishii et al. (2002), Zhao et al. (2012)
V. nashicola	2	China Yali 5	Chinese pear	Yali	Liaoning, China (1982)		Zhao et al. (2012)
V. nashicola	2	SD-YT-5	Chinese pear	Laiyang pear	Shandong, China (1995)		Zhao et al. (2012)
V. nashicola	2	Yali 05-2	Chinese pear	Yali	Liaoning, China (2005)	307111	
V. nashicola	2	Xingping 2	Chinese pear	Tanshan suli	Shaanxi, China (2006)	307120	Zhao et al. (2012)
V. nashicola	2	Anhui 2	Chinese pear	Dangshan suli	Anhui, China (2006)	307121	
V. nashicola	2	Yunnan 3	Chinese pear	Xuehua pear	Yunnan, China (2007)	307123	Zhao et al. (2012)
V. nashicola	4	Beijingbaili 4	Chinese pear	Jingbaili	Liaoning, China (2005)		Zhao et al. (2012)
V. nashicola	4	Jingbaili 05-2	Chinese pear	Jingbaili	Liaoning, China (2005)		
V. nashicola	5	F-1	Taiwanese pear	Hengshanli	Taichung, Taiwan (2005)	307113	Zhao et al. (2012)
V. nashicola	6	Jilin 1-4	Chinese pear	Nanguoli	Jilin, China (2007)	307125	Zhao et al. (2012)
V. nashicola	7	Shuibianli 1-1	Chinese pear	Shuibianli	Yunnan, China (2009)		Zhao et al. (2012)
V. nashicola	ND (8?)	Jingfengli 3	Chinese pear	Jingfengli	Liaoning, China (2005)	307109	Zhao et al. (2012)
V. nashicola	ND (9?)	Cili 5	Chinese pear	Chili	Liaoning, China (2005)	307110	Zhao et al. (2012)
V. nashicola	ND (10?)	Cili 7	Chinese pear	Chili	Liaoning, China (2005)	307112	Zhao et al. (2012)
V. nashicola	ND	JS-2	Japanese pear	Kousui	Kanagawa, Japan (1977)	306979	
V. nashicola	ND	Jingfengli 1-1	Chinese pear	Jingfengli	Liaoning, China (2005)		
V. nashicola	ND	Jingfengli 1-3	Chinese pear	Jingfengli	Liaoning, China (2005)		
V. nashicola	ND	Jingfengli 1-4	Chinese pear	Jingfengli	Liaoning, China (2005)		
V. nashicola	ND	Jingfengli 1-5	Chinese pear	Jingfengli	Liaoning, China (2005)		
V. nashicola	ND	Cili 1-2	Chinese pear	Chili	Liaoning, China (2005)		
V. nashicola	ND	Cili 1-3	Chinese pear	Chili	Liaoning, China (2005)		
V. nashicola	ND	Cili 1-4	Chinese pear	Chili	Liaoning, China (2005)		
V. nashicola	ND	Cili 1-5	Chinese pear	Chili	Liaoning, China (2005)		
V. nashicola	ND	Xingping 1	Chinese pear	Tanshan suli	Shaanxi, China (2006)		
V. nashicola	ND	Xingping 3	Chinese pear	Tanshan suli	Shaanxi, China (2006)		
V. nashicola	ND	Shandong 1-1	Chinese pear	Tse-pear	Shandong, China (2006)		
V. nashicola	ND	Shandong 1-2	Chinese pear	Tse-pear	Shandong, China (2006)	307118	Zhao et al. (2012)
V. nashicola	ND	Shandong 1-3	Chinese pear	Tse-pear	Shandong, China (2006)		
V. nashicola	ND	Shandong 4-1	Chinese pear	Ya-pear	Shandong, China (2006)		
V. nashicola	ND	Shandong 4-2	Chinese pear	Ya-pear	Shandong, China (2006)	307119	
V. nashicola	ND	Anhui 1	Chinese pear	Dangshan suli	Anhui, China (2006)		Zhao et al. (2012)
V. nashicola	ND	Anhui 3	Chinese pear	Dangshan suli	Anhui, China (2006)		
V. nashicola	ND	Anhui 4	Chinese pear	Dangshan suli	Anhui, China (2006)	307122	Zhao et al. (2012)
V. nashicola	ND	Yunnan 5	Chinese pear	Xuehua pear	Yunnan, China (2007)		
V. nashicola	ND	Jilin 1-1	Chinese pear	Nanguoli	Jilin, China (2007)		
V. nashicola	ND	Jilin 1-2	Chinese pear	Nanguoli	Jilin, China (2007)		
V. nashicola	ND	Jilin 2-1	Chinese pear	Balixing	Jilin, China (2007)		
V. nashicola	ND	Jilin 2-3	Chinese pear	Balixing	Jilin, China (2007)		
V. nashicola	ND	Jilin 3-1	Chinese pear	Pingxiangli	Jilin, China (2007)		
V. nashicola	ND	Jilin 3-2	Chinese pear	Pingxiangli	Jilin, China (2007)		
V. nashicola	ND	Jilin 3-3	Chinese pear	Pingxiangli	Jilin, China (2007)	307126	Zhao et al. (2012)
V. nashicola	ND	Jilin 4-1	Chinese pear	Xiehuatian	Jilin, China (2007)		
V. nashicola	ND	Jilin 4-2	Chinese pear	Xiehuatian	Jilin, China (2007)		
V. nashicola	ND	Jilin 4-3	Chinese pear	Xiehuatian	Jilin, China (2007)	307127	Zhao et al. (2012)
V. nashicola	ND	Luxi 1-1	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 1-2	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 1-3	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 1-4	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 2-1	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 2-2	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 2-3	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Luxi 2-4	Chinese pear	Xuehuali	Yunnan, China (2008)		
V. nashicola	ND	Housui 1	Japanese pear	Housui	Yunnan, China (2009)		
V. pyrina	Unknown	Akita SK-1	European pear	Starkrimson pear	Akita, Japan (1982)	615009	Zhao et al. (2012)
V. pyrina	Unknown	Shizukuishi FB-3	European pear	Flemish Beauty	Iwate, Japan (1983)		

[a] ND = not determined.

[b] Deposited at the National Agriculture and Food Research Organization Genetic Resources Center in Tsukuba, Ibaraki, Japan. MAFF = Ministry of Agriculture, Forestry, and Fisheries.

[c] Refer to Zhao et al. (2012) for GenBank accession numbers for the rDNA-ITS, β-tubulin, and elongation factor 1α gene sequences.

from Asian pears and ±1.79 and ±1.34 µm for conidia from European pear. These differences were consistent with values reported previously for *V. nashicola* conidia from Japanese pear (15.4 × 7.4 µm; range, 8.0 to 27.0 µm long and 5.1 to 11.8 µm wide) and from Chinese pear (15.8 × 7.6 µm; range, 8.3 to 26.3 µm long and 5.0 to 10.0 µm wide) (Ishii and Yanase 2000). In contrast, *V. pyrina* conidia from European pear were 22.0 × 7.8 µm (range, 9.5 to 42.4 µm long and 5.2 to 11.2 µm wide). Therefore, samples from Chinese and Taiwanese pears were identified morphologically as *V. nashicola*.

Mating experiments. The parental monoconidial isolates used in this study were all self-sterile (Tables 3, 4, 5, and 6), whereas pseudothecia and ascospores were formed in crossing the representative *V. nashicola* isolates from Japanese pear grown in Japan with those from the Asian pear strain Mamenashi 12 and the Chinese pear isolates (Table 3). Ascospores were also formed between isolates from Mamenashi 12 and those from Chinese pear and in crosses of the Chinese pear isolates (Table 3). Based on their mating compatibility, isolates were divided into two groups. Group 1 included OYO-1, JS-115, and Yasato 2-1-1 (all from Japan) and Jingfengli 1-4, Cili 1-3, Beijingbaili 3, Beijingbaili 5, Yali 05-1, Yali 05-2, Yali 05-3, and Yali 05-5 (all from Liaoning Province, China). Group 2 included Mamenashi 12A no. 1-1, Mamenashi 12A no. 1-3, Mamenashi 12A no. 1-4, and Mamenashi 12B no. 1-2 (all from Japan) and Cili 1-2, Cili 1-4, Cili 1-5, Jingfengli 1-1, Jingfengli 1-3, Jingfengli 1-5, Beijingbaili 4, and Yali 05-4 (all from Liaoning Province, China).

Isolates from Shaanxi Province in China produced ascospores when crossed with the isolate from Shandong Province and with the isolate from Anhui Province (Table 4). Shandong isolates also formed ascospores when crossed with Anhui isolates. In crosses of Shandong × Anhui isolates, most belonged to two groups. Group 1 comprised Xingping 1, Xingping 2, Xingping 3, Shandong 1-2, Shandong 4-1, Shandong 4-2, Anhui 1, Anhui 2, Anhui 3, and OYO-1. Group 2 comprised Shandong 1-1 and Shandong 1-3. Japanese pear isolate OYO-1 was fertile with isolate Shandong 1-1. Unexpectedly, isolate Anhui 4 produced ascospores when crossed with both groups of isolates classified (i.e., Xingping 1, Xingping 2, Shandong 1-2, Shandong 4-1, and Shandong 4-2 from group 1 and Shandong 1-3 from group 2). Isolate Anhui 4 from Anhui Province was unusual and also produced ascospores in the cross with *V. nashicola* isolate OYO-1 and *V. pyrina* isolate Akita SK-1 from Japan (Table 4).

Isolates collected from Jilin Province in China were fertile with the native Jilin isolates, and six isolates were also fertile with the isolates originating from Japanese pear grown in Japan (Table 5). Isolates were divided into two groups according to their mating compatibility. Group 1 comprised Jilin 4-2, Jilin 4-3, JS-115, JS-2, and OYO-1. Group 2 included Jilin 1-1, Jilin 1-2, Jilin 2-1, Jilin 2-3, Jilin 3-3, and Jilin 4-1. In this experiment, however, the unusual Chinese pear isolate Anhui 4 formed ascospores in the cross with three Japanese pear isolates of *V. nashicola* (JS-115, JS-2, and OYO-1) and two European pear isolates (Shizukuishi FB-3 and Akita SK-1) of *V. pyrina* derived from Japan. The ascospores thus formed were viable because they were observed to germinate on water agar (data not shown). The reproducibility of dual sexual compatibility with *V. nashicola* and *V. pyrina* isolates was thus confirmed for Anhui 4.

Ascospore formation was further confirmed in the cross of isolates from Yunnan Province, China, with a Japanese pear isolate and/or the other Yunnan isolates (Table 6). Isolate Housui 1, which originated from Japanese pear cultivar Housui grown in Yunnan, produced ascospores when crossed with Japanese isolates JS-115 and JS-2 (data not shown). Most importantly, isolate Yunnan 5 from Yunnan Province was fertile when crossed with *V. pyrina* isolate Akita SK-1 from European pear and produced viable ascospores in a separate experiment (data not shown).

Pathogenicity tests. *Inoculation with conidia from naturally occurring lesions.* In preliminary experiments, lesions sporulated profusely on Kousui leaves when conidial suspensions prepared

TABLE 2. Source and size of conidia of *Venturia* spp. collected from leaves or fruit on Chinese, Taiwanese, and European pear trees

				Length (µm)			Width (µm)		
Pear type	Time of sampling	Source location	Source cultivar	Range	Mean	SE	Range	Mean	SE
Chinese pear	9 August 2005	Liaoning, China	Jingfengli	7.9–21.2	15.7	0.34	5.2–9.7	7.0	0.10
	9 August 2005	Liaoning, China	Chili	9.8–21.3	15.5	0.34	5.2–8.3	6.8	0.10
	9 August 2005	Liaoning, China	Jingbaili	10.2–21.3	15.5	0.33	5.2–8.2	7.0	0.10
	9 August 2005	Liaoning, China	Yali	10.8–18.6	15.0	0.30	5.9–8.9	7.4	0.10
	31 July 2006	Shaanxi, China	Tanshan suli	6.4–15.6	11.6	0.29	3.7–9.3	6.9	0.20
	31 July 2006	Shaanxi, China	Tanshan suli	7.0–18.2	12.9	0.41	3.8–9.4	7.2	0.19
	31 July 2006	Shaanxi, China	Tanshan suli	9.0–21.0	15.0	0.35	5.1–14.7	8.1	0.18
	26 October 2006	Shandong, China	Tse-pear	12.0–20.0	15.0	0.26	6.8–11.3	9.2	0.13
	26 October 2006	Shandong, China	Tse-pear	10.1–21.7	16.0	0.38	7.3–11.6	8.9	0.13
	26 October 2006	Shandong, China	Tse-pear	12.6–23.5	17.8	0.30	7.1–11.0	9.0	0.13
	26 October 2006	Shandong, China	Ya-pear	13.6–23.9	18.1	0.36	7.4–11.9	9.0	0.17
	26 October 2006	Anhui, China	Dangshan suli	12.4–21.0	17.4	0.28	6.3–11.5	8.9	0.14
	5 June 2007	Yunnan, China	Xuehua	11.9–19.1	15.2	0.26	6.1–10.1	7.9	0.14
	11 October 2007	Jilin, China	Nanguoli	13.1–21.7	15.8	0.28	5.9–9.2	7.5	0.11
	11 October 2007	Jilin, China	Balixing	12.2–20.2	16.5	0.27	6.1–9.1	7.5	0.08
	11 October 2007	Jilin, China	Pingxiangli	13.5–20.7	17.5	0.29	6.1–8.5	7.3	0.09
	11 October 2007	Jilin, China	Xiehuatian	13.6–21,6	16.3	0.25	6.3–8.9	7.3	0.09
	3 June 2008	Yunnan, China	Xuehuali	13.7–22.0	17.2	0.27	7.3–10.4	8.6	0.12
	3 June 2008	Yunnan, China	Xuehuali	9.3–18.0	13.8	0.24	4.9–9.2	7.6	0.11
	17 August 2009	Yunnan, China	Meirensu	10.7–22.5	15.6	0.39	6.0–9.5	7.3	0.11
	17 August 2009	Yunnan, China	Shuibianli	10.8–25.8	16.9	0.47	5.2–11.7	8.8	0.18
	18 August 2009	Yunnan, China	Huobali	9.5–24.2	16.4	0.42	5.8–9.8	7.7	0.13
	18 August 2009	Yunnan, China	Huobali	10.4–20.8	15.5	0.33	5.7–9.9	7.7	0.14
	18 August 2009	Yunnan, China	Huobali	11.2–25.1	16.0	0.40	6.7–11.4	8.4	0.12
	19 August 2009	Yunnan, China	Yunhong 1 hao	7.9–22.5	15.1	0.48	5.3–9.2	7.0	0.11
	19 August 2009	Yunnan, China	Mantianhong	9.3–27.9	17.1	0.51	4.3–8.8	6.9	0.13
	19 August 2009	Yunnan, China	Housui	9.7–26.9	16.3	0.44	6.2–9.7	7.3	0.12
Taiwanese pear	12 July 2007	Taiwan	Hengshanli[a]	14.5–25.5	19.5	0.40	6.2–12.1	8.8	0.16
European pear	25 September 2007	Italy	William	16.8–27.1	21.8	0.38	7.4–11.8	9.0	0.13
	25 September 2018	Akita, Japan	Flemish Beauty	13.1–31.2	20.8	0.53	4.7–9.0	6.9	0.13
	9 October 2018	Hokkaido, Japan	Flemish Beauty	11.9–25.8	18.7	0.45	3.1–8.9	7.0	0.16

[a] Conidia produced after inoculation were measured.

TABLE 3. Ascospore formation by *Venturia nashicola* in compatibility tests of isolates from Japanese pear, Asian pear strain, and Chinese pear[a]

Isolate	OYO-1	JS-115	Yasato 2-1-1	Mamenashi 12A no. 1-1	Mamenashi 12A no. 1-3	Mamenashi 12A no. 1-4	Mamenashi 12B no. 1-2	Jingfengli 1-1	Jingfengli 1-3	Jingfengli 1-4
OYO-1[b]	–	–	–	–	–	–	–	+	–	–
JS-115		–	–	–	–	–	–	–	–	–
Yasato 2-1-1			–	–	–	+	–	–	–	–
Mamenashi 12A no. 1-1[c]				–	–	–	–	–	–	–
Mamenashi 12A no. 1-3					–	–	–	–	–	–
Mamenashi 12A no. 1-4						–	–	–	–	–
Mamenashi 12B no. 1-2							–	–	–	–
Jingfengli 1-1[d]								–	–	–
Jingfengli 1-3									–	–
Jingfengli 1-4										–
Jingfengli 1-5										
Cili 1-2										
Cili 1-3										
Cili 1-4										
Cili 1-5										
Beijingbaili 3										
Beijingbaili 4										
Beijingbaili 5										
Yali 05-1										
Yali 05-2										
Yali 05-3										
Yali 05-4										
Yali 05-5										

(*Continued on next page*)

[a] Plus signs indicate fertile, whereas minus signs indicate sterile.
[b] Isolates OYO-1, JS-115, and Yasato 2-1-1 are from Japanese pear grown in Japan.
[c] Isolates Mamenashi 12A no. 1-1, Mamenashi 12A no. 1-3, Mamenashi 12A no. 1-4, and Mamenashi 12B no. 1-2 are from an Asian pear strain grown in Japan.
[d] Isolates Jingfengli 1-1, Jingfengli 1-3, Jingfengli 1-4, Jingfengli 1-5, Cili 1-2, Cili 1-3, Cili 1-4, Cili 1-5, Beijingbaili 3, Beijingbaili 4, Beijingbaili 5, Yali 05-1, Yali 05-2, Yali 05-3, Yali 05-4, and Yali 05-5 are from Chinese pear grown in Liaoning Province, China.

from Kousui leaves sampled in Japan were used, whereas only chlorosis and tiny brown spots formed on Jingbaili leaves and stems, respectively (data not shown). This finding was consistent with results reported previously (Abe and Kurihara 1993; Ishii et al. 1992). Conidia were produced abundantly only on Jingbaili, which was inoculated with suspensions of conidia collected from leaves of Jingbaili grown in Liaoning, China. No conidia formed on the other pear trees (data not shown). Therefore, isolates collected from Jingbaili differed clearly in pathogenicity from isolates within Japan. Similarly, conidia collected from cultivars Nanguoli, Balixing, and Pingxiangli grown in Liaoning, China, exhibited distinct pathogenicity from that of known pathogenic races (data not shown), thus encouraging us to search for new races of this pathogen.

Inoculation with conidia produced in cellophane culture. Overall results from the inoculation experiments are summarized in Figure 1 and Tables 7 and 8. Race 1 isolate Yasato 2-1-1 was pathogenic on Japanese pear cultivar Kousui but not on Asian pear strain Mamenashi 12, as confirmed previously (Ishii et al. 2002). This isolate was also pathogenic on Chinese pear cultivar Yali but not on any other inoculated pear cultivars. Mamenashi 12A no. 1-3 and no. 1-4, which are known race 2 isolates (Ishii et al. 2002), were pathogenic on Mamenashi 12 and Yali but not on any other pear cultivars, including Kousui. Interestingly, isolates SD-YT-5, Yali 05-2, China Yali 5, Shandong 4-2, Anhui 2, Xingping 2, and Yunnan 3, which had the same pathogenicity on the differential hosts as the race 2 isolates, were isolated from pear grown in several Chinese provinces (including Liaoning, Shandong, Anhui, Shaanxi, and Yunnan) that are all geographically distant from each other (Figs. 1 and 2A). Race 3 isolate Mamenashi 12B no. 53-1, which was pathogenic to both Kousui and Mamenashi 12 (Ishii et al. 2002), was also pathogenic on Yali. Races 1 and 3 were not found among any tested isolates from China and Taiwan. Instead, several isolates that differed from known races 1, 2, and 3 of *V. nashicola* in their pathogenicity were isolated from pear grown in these regions.

Isolate Beijingbaili 4 from Liaoning Province, China, was pathogenic to Jingbaili, the original host cultivar of Chinese pear, and to Yali but not to the other cultivars. Isolate Beijingbaili 4 was thus designated race 4 because such differential pathogenicity was not described previously. Isolate Jingbaili 05-2 from Liaoning also showed the same differential pathogenicity as Beijingbaili 4 (Fig. 2B). Isolate F-1 from the Taiwanese pear cultivar Hengshanli was pathogenic only on this original host cultivar (Fig. 2C) and was thus designated race 5. Isolate Jilin 1-4 from Jilin Province, China, formed conidia only on Chinese pear cultivars Jingbaili and Nanguoli (race 6) (Fig. 2D). Yunnan isolate Shuibianli 2 was pathogenic only on the representative Chinese pear Yali (race 7). Three isolates from Liaoning Province (Jingfengli 3, Cili 5, and Cili 7) were pathogenic to Mamenashi 12, Yali, and Linyuli (Fig. 2E) but differed in their pathogenicity on Jingbaili and Nanguoli. Isolate Jingfengli 3 produced conidia on Mamenashi 12 and Jingbaili, Yali, Linyuli, and Nanguoli. Isolate Cili 5 also produced conidia on Mamenashi 12 and Jingbaili, Yali, and Linyuli but not on Nanguoli. In contrast, isolate Cili 7 was pathogenic on Mamenashi 12, Yali, Linyuli, and Nanguoli but not on Jingbaili. Therefore, isolates Jingfengli 3, Cili 5, and Cili 7 are potentially representative of new races 8, 9, and 10, respectively, but we left them unspecified because these results could not be confirmed in subsequent experiments (Table 7).

Among 10 pear cultivars or genotypes inoculated with 19 monoconidial isolates, Yali was highly susceptible to 16 isolates (Fig. 2F), but no visible symptoms developed on nonhost European pear cultivar Flemish Beauty (Table 7). Japanese pear cultivar Kinchaku and *P. sinkiangensis* 'Xiangli' were also highly resistant to all isolates tested and did not develop scab symptoms (Figs. 3 and 4; Table 7).

Potential role of sexual reproduction in the emergence of new races. Of the 36 single-ascospore isolates from the cross between race 1 (Yasato 2-1-1) and race 2 (Mamenashi 12A no. 1-4) isolates, lesions of three isolates sporulated on leaves of Kousui but not on Mamenashi 12, as did the parental race 1 isolate. Fourteen isolates sporulated on Mamenashi 12 but not on cultivar Kousui leaves, equivalent to the parental race 2 isolate. However, the other four isolates had dual pathogenicity on both Kousui and Mamenashi 12, characteristic

TABLE 3. (*Continued from previous page*)

Jingfengli 1-5	Cili 1-2	Cili 1-3	Cili 1-4	Cili 1-5	Beijingbaili 3	Beijingbaili 4	Beijingbaili 5	Yali 05-1	Yali 05-2	Yali 05-3	Yali 05-4	Yali 05-5
−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	+	−
−	−	−	−	+	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	+	−	−	−	+
−	−	−	−	−	−	−	−	+	+	+	−	−
−	−	−	−	−	−	−	−	+	+	−	−	+
−	−	−	−	−	−	−	−	+	+	−	−	−
−	−	−	−	−	−	−	+	+	−	−	−	+
−	−	−	−	−	−	−	−	+	+	−	−	+
−	+	−	−	+	−	−	−	−	−	−	+	−
−	−	−	−	−	−	−	−	−	+	−	−	−
	−	+	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−	−
			−	−	−	−	−	+	−	−	−	+
				−	+	−	−	−	+	+	−	+
					−	−	−	−	−	−	−	−
						−	−	+	−	−	−	−
							−	−	−	−	−	−
								−	−	−	−	−
									−	−	+	−
										−	−	−
											−	−
												−

TABLE 4. Ascospore formation by *Venturia nashicola* and *V. pyrina* in compatibility tests of isolates from Japanese pear, Chinese pear, and European pear[a]

Isolate	Xingping 1	Xingping 2	Xingping 3	Shandong 1-1	Shandong 1-2	Shandong 1-3	Shandong 4-1	Shandong 4-2	Anhui 1	Anhui 2	Anhui 3	Anhui 4	OYO-1	Akita SK-1
Xingping 1[b]	−	−	−	−	−	+	−	−	−	−	−	+	−	−
Xingping 2		−	−	−	−	+	−	−	−	−	−	+	−	−
Xingping 3			−	−	−	+	−	−	−	−	−	−	−	−
Shandong 1-1				−	−	−	−	−	+	−	−	−	+	−
Shandong 1-2					−	−	−	−	−	−	−	+	−	−
Shandong 1-3						−	−	+	+	+	+	+	−	−
Shandong 4-1							−	−	−	−	−	+	−	−
Shandong 4-2								−	−	−	−	+	−	−
Anhui 1									−	−	−	−	−	−
Anhui 2										−	−	−	−	−
Anhui 3											−	−	−	−
Anhui 4												−	+	+
OYO-1[c]													−	−
Akita SK-1[d]														−

[a] Plus signs indicate fertile, whereas minus signs indicate sterile.
[b] Isolates Xingping 1, Xingping 2, Xingping 3, Shandong 1-1, Shandong 1-2, Shandong 1-3, Shandong 4-1, Shandong 4-2, Anhui 1, Anhui 2, Anhui 3, and Anhui 4 are from Chinese pear grown in Shaanxi, Shandong, and Anhui provinces, China.
[c] Isolate OYO-1 is from Japanese pear grown in Japan.
[d] Isolate Akita SK-1 is from European pear grown in Japan.

of race 3 isolates. The remaining 15 isolates were not pathogenic on either of the two differential hosts. Segregation of the progenies did not fit a 1:1 (race 1/race 2) ratio in this experiment.

DISCUSSION

Determining the breadth of *V. nashicola* pathogenic specialization is necessary to avoid breakdown of scab resistance in pear hosts. Research is hampered by extreme difficulty in producing *V. nashicola* conidia in culture, which is even more challenging than *V. inaequalis*, a poor sporulator in culture (Parker et al. 1995). Sporulation in planta requires a long incubation period of at least 3 to 4 weeks under optimal conditions. These challenges likely contribute to reasons why only races 1, 2, and 3 have been characterized among Japanese isolates of *V. nashicola* (Ishii et al. 2002). As a result, the broader pathogenic specialization of this important fungus is only now being reported.

In this study, *Venturia* isolates were collected from Asian pears grown in China and Taiwan to evaluate conidial size, mating compatibility in culture, and pathogenicity on leaves of potted pear trees in comprehensive experiments over a 10-year period. All isolates were identified as *V. nashicola* based on conidial size compared with reported data (Ishii and Yanase 2000). These results are consistent with our previous phylogenetic studies (Zhao et al. 2012, 2016). Isolates collected from Japanese, Chinese, and Taiwanese pears formed a distinct evolutionary lineage from those derived from European pear, and this result corroborates the early taxonomic separation of *V. nashicola* from *V. pyrina* (Ishii and Yanase 2000; Tanaka and Yamamoto 1964) and the separation among *Venturia* spp. described based on the *TUB2* gene sequence (Bock et al. 2021).

Clear sexual isolation between *V. nashicola* and *V. pyrina* was confirmed previously in mating experiments (Ishii and Yanase 2000). In this study, representative isolates of *V. nashicola* (races 1, 2, and 3) from Japanese pear and an Asian pear strain formed ascospores with Chinese pear isolates from various distantly separated regions in China, indicating that isolates of pear scab from regions across China were also *V. nashicola*. Ascospores were produced between crosses of Chinese pear isolates, indicating that the isolates of both mating types are present in China. *V. nashicola* is a heterothallic species. Surprisingly, an isolate from Anhui Province (Anhui

4) in China produced ascospores when crossed with isolates belonging to mating groups I and II. Anhui 4 produced pseudothecia and formed ascospores when crossed with one of the three Japanese pear isolates and with the two European pear isolates, all isolated from Japan. This is the first report of pear isolates having dual sexual compatibility with both *V. nashicola* and *V. pyrina*, although all isolates used in this study were obtained by single-spore isolation and were self-sterile. A similar phenomenon was reported for two other heterothallic fungi, *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* (causal agents of net blotch on barley), which formed natural hybrids after sexual recombination between isolates of opposite mating types in the field (Poudel et al. 2017). Budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*) both switch-on mating types during vegetative growth by homologous recombination repair of the expressed mating-type locus (Thon et al. 2019). These phenomena have not been described previously for a *Venturia* species. However, *V. pyrina* was shown to produce sexual structures on its nonhost apple when *V. pyrina* ascospores were isolated from apple leaf litter (Stehmann et al. 2001). These observations suggest that further detailed characterization of *V. nashicola* and *V. pyrina* mating systems is required.

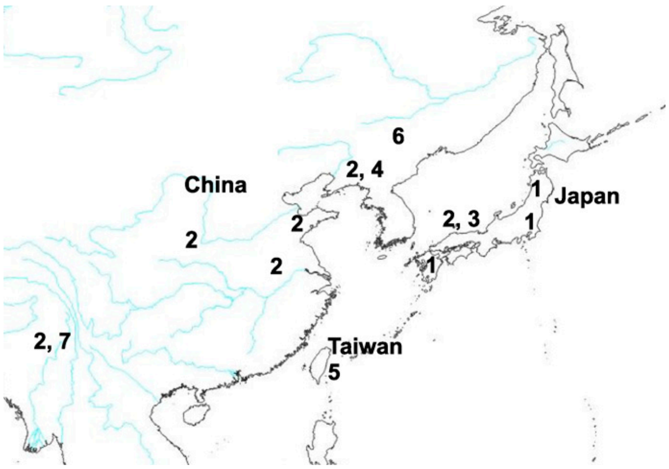

Fig. 1. Distribution of pathogenic races 1 to 7 of *Venturia nashicola* in East Asia.

The pathogenicity of individual *V. nashicola* isolates collected from China and Taiwan, in addition to the Japanese isolates used as a reference, was examined qualitatively based on conidial production, rather than chlorosis and/or necrosis symptoms, on inoculated leaves of potted pear trees. In our previous study (Ishii et al. 2002), Japanese pear cultivar Kousui and Asian pear strain Mamenashi 12 were used to distinguish *V. nashicola* races. However, in this study, Chinese pear cultivars Jingbaili, Yali, Linyuli, and Nanguoli and Taiwanese pear cultivar Hengshanli were used as differential hosts in addition to Kousui and Mamenashi 12.

Race 1 isolates were pathogenic to Kousui and Yali. Race 2 isolates were pathogenic on Mamenashi 12 and Yali. Race 3 isolates were pathogenic to cultivars Kousui and Yali and to Mamenashi 12. All isolates belonging to these three races were nonpathogenic on the remaining inoculated pear cultivars. Adachi et al. (2004) reported that Chinese pear cultivar Nanguoli was susceptible to race 2 and race 3 isolates. The reason for this discrepancy in results

TABLE 5. Ascospore formation by *Venturia nashicola* and *V. pyrina* in compatibility tests of isolates from Japanese pear, Chinese pear, and European pear[a]

Isolate	Jilin 1-1	Jilin 1-2	Jilin 2-1	Jilin 2-3	Jilin 3-3	Jilin 4-1	Jilin 4-2	Jilin 4-3	Anhui 4	JS-115	JS-2	OYO-1	Shizukuishi FB-3	Akita SK-1
Jilin 1-1[b]	–	–	–	–	–	–	+	–	–	+	+	+	–	–
Jilin 1-2		–	–	–	–	–	–	–	–	–	+	+	–	–
Jilin 2-1			–	–	–	–	–	–	–	–	+	–	–	–
Jilin 2-3				–	–	–	+	–	–	+	–	–	–	–
Jilin 3-3					–	–	–	–	–	+	+	–	–	–
Jilin 4-1						–	–	+	–	+	+	+	–	–
Jilin 4-2							–	–	–	–	–	–	–	–
Jilin 4-3								–	–	–	–	–	–	–
Anhui 4									–	+	+	+	+	+
JS-115[c]										–	–	–	–	–
JS-2											–	–	–	–
OYO-1												–	–	–
Shizukuishi FB-3[d]													–	–
Akita SK-1														–

[a] Plus signs indicate fertile, whereas minus signs indicate sterile.
[b] Isolates Jilin 1-1, Jilin 1-2, Jilin 2-1, Jilin 2-3, Jilin 3-3, Jilin 4-1, Jilin 4-2, Jilin 4-3, and Anhui 4 are from Chinese pear grown in Jilin and Anhui provinces, China.
[c] Isolates JS-115, JS-2, and OYO-1 are from Japanese pear grown in Japan.
[d] Isolates Shizukuishi FB-3 and Akita SK-1 are from European pear grown in Japan.

TABLE 6. Ascospore formation by *Venturia nashicola* in compatibility tests of isolates from Japanese pear and Chinese pear[a]

Isolate	Luxi 1-1	Luxi 1-2	Luxi 1-3	Luxi 1-4	Luxi 2-1	Luxi 2-2	Luxi 2-3	Luxi 2-4	JS-2
Luxi 1-1[b]	–	–	–	–	–	–	–	–	+
Luxi 1-2		–	–	–	+	+	+	+	+
Luxi 1-3			–	–	+	+	+	+	+
Luxi 1-4				–	+	+	+	+	–
Luxi 2-1					–	–	–	–	–
Luxi 2-2						–	–	–	–
Luxi 2-3							–	–	–
Luxi 2-4								–	–
JS-2[c]									–

[a] Plus signs indicate fertile, whereas minus signs indicate sterile.
[b] Isolates Luxi 1-1, Luxi 1-2, Luxi 1-3, Luxi 1-4, Luxi 2-1, Luxi 2-2, Luxi 2-3, and Luxi 2-4 are from Chinese pear grown in Yunnan Province, China.
[c] Isolate JS-2 is from Japanese pear grown in Japan.

remains to be clarified. Importantly, we identified the existence of four additional *V. nashicola* races (races 4, 5, 6, and 7), which are described here for the first time. Race 4 is pathogenic on Chinese pear cultivar Jingbaili, the original host of the isolates, and on cultivar Yali. Race 5 is pathogenic only on Taiwanese pear cultivar Hengshanli, the original host of the isolates. Race 6 is pathogenic on Chinese pear cultivars Jingbaili and Nanguoli. Race 7 is pathogenic only on cultivar Yali. Yali was highly susceptible to most of the isolates belonging to the existing and newly described races, excluding race 5 from Taiwan and race 6 from Jilin Province in China. Interestingly, isolates of race 1 and race 3 were not detected in China or Taiwan, and none of the four newly found races were pathogenic to cultivar Kousui.

In our previous phylogenetic analysis, three Taiwanese isolates, including one used in this study, were grouped in the same clade as *V. nashicola* isolates (Zhao et al. 2012). The phylogenetic position and the limited host range of race 5 isolates (i.e., pathogenic only on cultivar Hengshanli, which is grown extensively in Taiwan) (Teng et al. 2002) may reflect geographical isolation of pathogenic races of *V. nashicola*. In a random amplified polymorphic DNA (RAPD) analysis, cultivar Hengshanli, which is considered to have been introduced from southern China, was subclustered with a cultivar native to Fujian Province, China (Teng et al. 2002). Cultivars Jingbaili and Nanguoli were clustered together, indicating a close relationship between them. *V. nashicola* races that were pathogenic to cultivars Jingbaili, Linyuli, and Nanguoli were all from the northern region of China, possibly as a result of selection for these races on pear cultivars in these areas. Although we did not group some of the isolates from Liaoning as a new race here, their pathogenicity pattern on the differential hosts was unique; however, the results need to be reproduced and confirmed. Furthermore, various wild pear species also develop scab, and we previously isolated *V. nashicola* from some of these species (Ishii et al. 2002). It is quite possible that more new races of *V. nashicola* will be found in the future.

V. nashicola race 1 isolates are dominant in Japanese pear orchards, whereas race 2 isolates have been reported only from Tottori Prefecture in western Japan (Ishii et al. 2002). Race 2 isolates were detected from several provinces in China, indicating that they may be endemic to China and transported or dispersed to western Japan. Interestingly, race 3 isolates were also found in Tottori (Ishii et al. 2002). Race 3 might have emerged through recombination of pathogenicity factors between race 1 and race 2 in a natural cross in the field, because the race 2 isolates were sampled from a Mamenashi 12 tree growing in isolation from other pear trees in the field. In comparison, race 3 isolates were from a Mamenashi 12 tree grown among a mixture of susceptible Japanese pear cultivars all in close proximity in the experimental pear orchard. To test this hypothesis, we crossed a monoconidial isolate of race 1 and race 2 on culture medium, and the resultant single-ascospore isolates were used to inoculate Kousui and Mamenashi 12 trees. As expected, in addition to the parental race 1-type and race 2-type isolates, race 3-type progeny isolates were generated.

Race 1 and race 3 isolates have not yet been found in China, suggesting the possibility that race 3 isolates emerged as hypothesized through a recombination of virulence genes between race 1 and race 2 isolates. The difference in host range might also have been influenced by a recombination of virulence genes. In the agricultural environment, strong selection can aid the adaptation of a pathogen to new environmental factors, including host genotypes (Corredor-Moreno and Saunders 2020). Pear species originated in the western parts of China and moved either eastward or westward, followed by the geographical differentiation of Asian pears and European pear (Saito 2016). Species-specific RAPD markers were designed and a clustering analysis revealed two divisions, one comprising *P. communis* cultivars and the other including *Pyrus*

TABLE 8. Response of differential hosts of Japanese pear, Asian pear strain, and Chinese pear to *Venturia nashicola* races[a]

	Differential host						
Race	Kousui	Mamenashi 12	Jingbaili	Hengshanli	Yali	Linyuli	Nanguoli
1	+	–	–	–	+	–	–
2	–	+	–	–	+	–	–
3	+	+	–	–	+	–	–
4	–	–	+	–	+	–	–
5	–	–	–	+	–	–	–
6	–	–	+	–	–	–	+
7	–	–	–	–	+	–	–

[a] Plus signs indicate susceptible (sporulation), whereas minus signs indicate resistant.

TABLE 7. Incidence of sporulating lesions of scab (caused by *Venturia nashicola*) on Japanese pear, Asian pear strain, Chinese pear, and Taiwanese pear after inoculation with isolates of different races from Japan, China, and Taiwan[a]

Race	Isolate	Kousui	Mamenashi 12	Jingbaili	Hengshanli	Yali	Linyuli	Nanguoli	Kinchaku	Xiangli	Flemish Beauty
1	Yasato 2-1-1	66.7/22.2	0/0	0/0	0/0	66.7/86.7	0/0	0/0	–/–	0/0	0/–
2	Mamenashi 12A no. 1-3	0/0	100/100	0/0	0/0	60.0/100	0/0	0/0	–/–	0/0	0/–
2	Mamenashi 12A no. 1-4	0/0	100/100	0/0	0/0	100/100	0/0	0/0	–/–	0/0	0/–
2	SD-YT-5	0/0	100/50.0	0/0	0/0	100/0.0	0/0	0/0	0/0	0/0	0/0
2	Yali 05-2	0/0	13.3/100	0/0	0/0	100/100	0/0	0/0	0/0	0/0	0/–
2	China Yali 5	0/0	33.3/40.0	0/0	0/0	88.9/20.0	0/0	0/0	0/0	0/0	0/–
2	Shandong 4-2	0/0	100/8.3	0/0	0/0	100/26.7	0/0	0/0	0/0	0/–	0/0
2	Anhui 2	0/0	88.9/13.3	0/0	0/0	100/0.0	0/0	0/0	0/0	0/–	0/0
2	Xingping 2	0/0	66.7/13.3	0/0	0/0	44.4/13.3	0/0	0/0	0/–	0/–	0/–
2	Yunnan 3	0/0	80.0/100	0/0	0/0	90.0/66.7	0/0	0/0	0–	0/–	0/–
3	Mamenashi 12B no. 53-1	77.8/–	100/44.4	0/0	0/0	60.0/86.7	0/0	0/0	0/–	0/0	0/–
4	Beijingbaili 4	0/0	0/0	100/66.7	0/0	100/73.3	0/0	0/0	0/0	0/0	0/0
4	Jingbaili 05-2	0/0	0/0	100/13.3	0/0	0/66.7	0/0	0/0	0/0	0/–	–/–
5	F-1	0/0	0/0	0/0	66.7/0	0/0	0/0	0/0	0/0	0/–	0/0
6	Jilin 1-4	0/0	0/0	100/66.7[b]	0/0	0/0	0/0	100/66.7[b]	0/0	0/–	0/–
7	Shuibianli 2	0/0	0/0	0/0	0/0	100/100	0/0	0/0	0/0	0/–	0/0
Undetermined (8?)	Jingfengli 3	0/0	100/100	100/73.3	0/0	100/100	44.4/0	0/33.3	0/0	0/0	0/–
Undetermined (9?)	Cili 5	0/0	86.7/40.0	20.0/66.7	0/0	93.3/50.0	22.2/0	0/0	0/0	0/–	0/0
Undetermined (10?)	Cili 7	0/0	86.7/73.3	0/0	0/0	86.7/66.7	33.3/0	0/33.3	0/0	0/–	0/0

[a] Incidence (%) of sporulating lesions = (number of sporulating sites/number of drop-inoculated sites) × 100. Values from two independent trials are shown. Dashes indicate not tested because not enough conidia were produced in cellophane culture.

[b] Conidia formed on inoculated Nanguoli leaves were used as inoculum in the second trial.

accessions native to East Asia (Teng et al. 2002). These changes were accompanied by the coevolution of *V. nashicola* on Asian pears and *V. pyrina* on European pear. In our inoculation tests, European pear cultivar Flemish Beauty, which is highly susceptible to *V. pyrina* in Japan, was highly resistant to various isolates of *V. nashicola* as expected, because European pear is a nonhost of *V. nashicola* (Abe et al. 2008; González-Domínguez et al. 2017; Ishii and Yanase 2000; Tanaka and Yamamoto 1964).

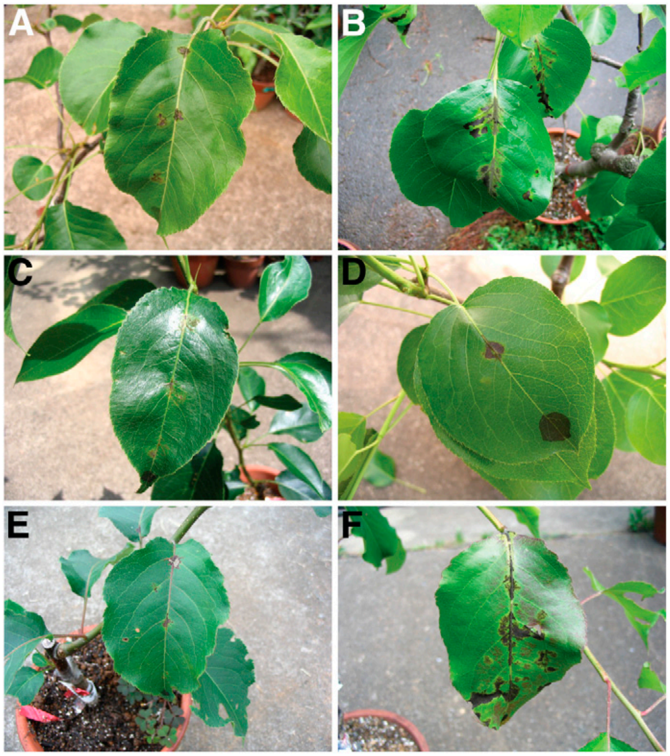

Fig. 2. Sporulating lesions of scab caused by *Venturia nashicola* on pear leaves 1 month after inoculation with isolates from China and Taiwan. **A,** Race 2 isolate Yunnan 4 from Yunnan Province, China, on Asian pear strain Mamenashi 12. **B,** Race 4 isolate Jingbaili 05-2 from Liaoning Province, China, on Chinese pear cultivar Jingbaili. **C,** Race 5 isolate F-1 from Taiwan on Taiwanese pear cultivar Hengshanli. **D,** Race 6 isolate Jilin 1-4 from Jilin Province, China, on Chinese pear cultivar Nanguoli. The black spot on the margin of the inoculated leaf was made using a felt-tip marker. **E,** Isolate Cili 7 (race undetermined) from Liaoning Province, China, on Chinese pear cultivar Linyuli. **F,** Race 4 isolate Beijingbaili 4 from Liaoning Province, China, on Chinese pear cultivar Yali.

Japanese pear cultivar Kinchaku was resistant to isolates sampled from Japan, various regions of China, and Taiwan, which is in agreement with previous observations that Kinchaku is highly resistant to *V. nashicola* (Abe and Kurihara 1993; Ishii et al. 1992). The resistance of Kinchaku was deployed subsequently in a scab resistance breeding program, leading to the registration and release of novel Japanese pear cultivar Hoshiakari (Saito 2016). Hoshiakari resulted from a cross between strain 314-32 (Kinchaku × Housui) and Japanese pear cultivar Akiakari and is highly resistant to both scab and black spot (Saito et al. 2015). Moreover, the new interspecific pear cultivar Yutaka is resistant to *V. nashicola* races 1, 2, and 3 and two other pathogens (Ishii and Kimura 2018). Korla fragrant pear (*P. sinkiangensis*), native to the southern part of the Xinjiang Uyghur Autonomous Region in China, has been cultivated for nearly 1,400 years (Zhou et al. 2018). Cultivar Xiangli is particularly popular in China; however, no reliable reports are available for scab symptoms on Xiangli in grower orchards. We confirmed that Xiangli is scab resistant and has potential to be useful for scab resistance breeding.

Although further research on host and pathogen genotypes and interactions is required, the interaction of *V. nashicola* with differential hosts might follow a “gene-for-gene” concept, with the recognition of race-specific secreted effector proteins (Avr factors) mediated by host genotype-specific immune sensors (resistance proteins) (Cesari 2018). Analysis of genomic sequences is expected to help elucidate the evolutionary history of *Venturia* species on Rosaceae host crops, including pear (Le Cam et al. 2019). The whole genome sequence of *V. nashicola* was published in 2019 (Johnson et al. 2019; Prokchorchik et al. 2019). Prokchorchik et al. (2020) further analyzed the genome using *V. nashicola* and *V. pyrina* isolates to identify the core effector repertoire and potential effectors specific to *V. nashicola*, although the race of the two isolates used was not described. Prokchorchik et al. (2020) suggested that these effectors may act as host-range determinants between *V. nashicola* and *V. pyrina*, enabling avirulence recognition in nonhost plant species. The pathogenic specialization of the *V. nashicola* isolates used in this study has been well characterized. Therefore, they will be useful for finding candidate effectors from individual races and aid our understanding of their evolutionary history and interactions with host and nonhost pear species.

Fig. 3. Scab resistance (no symptoms) observed on leaves of Japanese pear cultivar Kinchaku 1 month after inoculation with *Venturia nashicola* race 1 isolate Yasato 2-1-1 from Ibaraki Prefecture, Japan.

Fig. 4. Scab resistance (no symptoms) observed on leaves of *Pyrus sinkiangensis* ‘Xiangli’ (Korla fragrant pear) 1 month after inoculation with *Venturia nashicola* race 2 isolate Shandong 4-2 from Shandong Province, China. The black spot on the margin of the inoculated leaf was made using a felt-tip marker.

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