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A new interspecific pear cultivar Yutaka: highly resistant to the two major diseases scab and black spot on Asian pears

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Abstract Scab, caused by *Venturia nashicola*, is the most important disease of Asian pears. In Japan, the most popular and widely grown pear cultivar (cv.) Kousui is resistant to black spot, caused by the Japanese pear pathotype of *Alternaria alternata*, but highly susceptible to scab. Recently, a new interspecific pear cv. Yutaka carrying high fruit quality was registered officially after a cross between the Chinese pear cv. Hongli and the Japanese pear cv. Housui. Accordingly, tests were conducted to assess resistance of cv. Yutaka to scab, black spot and anthracnose. When conidial suspensions of *V. nashicola* were spray inoculated onto potted trees, leaves of cv. Yutaka showed no disease symptoms although severe scab development was recorded on the susceptible reference cv. Kousui. Furthermore, neither black spot symptoms nor necrosis formation were observed when leaves of cv. Yutaka were inoculated with conidial suspensions of the *A. alternata* Japanese pear pathotype or treated with a culture filtrate containing the host-specific AK-toxin of this pathogen, respectively. These results strongly

indicate that cv. Yutaka is highly resistant to scab and black spot. Inoculation tests also suggested the potential high levels of resistance of cv. Yutaka to anthracnose caused by *Colletotrichum gloeosporioides* sensu lato. The introduction of this new pear cultivar will reduce fungicide applications in pear orchards and thus lower the risk of fungicide resistance development in these pathogens.

Keywords Anthracnose · Black spot · Disease resistance · Pear · Scab

In deciduous tree fruits, worldwide pear production is second to that of apples (Saito 2016). Scab caused by *Venturia nashicola*, is the most important disease of Japanese pear (*Pyrus pyrifolia* var. *culta*) and Chinese pear (*P. ussuriensis* and *P. bretschneideri*), all grown commercially in East Asia (Ishii and Yanase 2000). Asian pear scab is a quarantine disease in many countries (Australian Quarantine and Inspection Service 1998; Le Cam et al. 2001; Koh et al. 2013). In Japan, cv. Kousui is the most popular and widely grown pear cultivar. It is highly susceptible to scab, but resistant to black spot caused by the Japanese pear pathotype of *Alternaria alternata*. Since there are few scab-resistant cultivars acceptable to growers, frequent fungicide applications are required to control the disease. As a result, strains of *V. nashicola* resistant to benzimidazole and/or DMI (sterol demethylation inhibitor) fungicides have been widely distributed (Ishii et al. 1992b; Ishii 2012). The two scab-susceptible Japanese pear cvs Housui and

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Niitaka are widely grown, and they are quite susceptible to anthracnose caused by *Colletotrichum gloeosporioides* sensu lato (Tashiro et al. 2012) as well. This pathogen has also developed fungicide resistance reducing the control efficacy of fungicides (Chung et al. 2006; Tashiro et al. 2012). Under such circumstances, multiple disease resistance has become a very important objective in pear breeding (Saito 2016).

Pear cv. Yutaka (Fig. 1) was bred by the private pear grower Y. Kimura and registered officially (No. 17789) in March 2008 by the Ministry of Agriculture, Forestry and Fisheries in Japan. The cultivar was selected from the progenies of the Chinese pear cv. Hongli and the scab susceptible cv. Housui. Cv. Yutaka has high fresh weight and characteristics highly acceptable to consumers. Due to these reasons, it has already been introduced in commercial pear orchards in some regions of Japan. Cv. Hongli, one of the parents of cv. Yutaka, was found to be highly resistant to scab (Ishii et al. 1992a; Abe and Kurihara 1993), and subsequent genetic analyses indicated that the scab resistance of cv. Hongli is dominant (Abe and Kotobuki 1998a, b). The resistance of cv. Hongli to three races of *V. nashicola* was briefly reported by Adachi et al. (2004). In addition, full resistance of the parental cvs. Hongli and Housui to black spot disease was reported by Kozaki (1973). The objective of this study was to test the resistance of cv. Yutaka to scab, black spot and anthracnose.

In *V. nashicola*, three pathological races were found to have differential pathogenicity in Japanese pear cv. Kousui and Asian pear strain Mamenashi 12 (species unknown) (Ishii et al. 2002). Race 1 isolates are pathogenic to cv. Kousui, and race 2 isolates are pathogenic to

strain Mamenashi 12, whereas race 3 isolates are pathogenic to both cv. Kousui and strain Mamenashi 12. In this study, fresh conidia of races 1 and 3 of *V. nashicola*, collected directly from naturally occurring scab lesions on pear leaves, were used as inoculum. Separately, monoconidial stock isolates of *V. nashicola*, two of each race, were employed for conidial production on cellophane culture (Ishii et al. 2002). For inoculation experiments (Table 1), potted trees of cvs Yutaka, Kousui and strain Mamenashi 12 were used. In addition, 23 young seedlings derived from a cross of scab susceptible cv. Natsushizuku with cv. Yutaka and a parent cv. Natsushizuku were grown in pots and used in the test (Fig. 2). Potted trees of cv. Kousui and strain Mamenashi 12, both susceptible to race 3 of *V. nashicola*, were also used as a reference. Conidial suspensions of *V. nashicola* ($2.5\text{--}5 \times 10^5$ conidia mL⁻¹, with 0.1% sucrose added) were spray inoculated using a hand-held sprayer onto actively growing shoots with fresh leaves susceptible to scab. Inoculated plants were incubated in a moist chamber at 20 °C for 48 h and transferred to either a screen-house outdoor (approximately 15 to 25 °C) or a phytotron maintained at 25 °C. One month after inoculation, the percentage (%) of leaves with sporulating lesions (incidence) was recorded together with disease severity calculated according to Ishii et al. (1992a). Briefly, scab development on each leaf was scored based on the degree of sporulation as follows: 4, abundantly sporulating; 3, moderately or sparsely sporulating; 2, chlorotic or necrotic lesions with no sporulation; 1, pinhole with no sporulation; and 0, no visible symptoms. Disease severity was calculated as $([4A + 3B + 2C + 1D]/4M) \times 100$, where A, B, C, and D is the number of leaves corresponding to the scales 4, 3, 2, and 1, respectively, and M is the total number of leaves assessed.

Values shown below are means and the 95% confidence intervals from three replicate plants. Suspensions of conidia, prepared from pure cellophane culture, were drop inoculated onto the midribs of leaves. For three portions for each of the three leaves, the percentage of the sporulating portions was calculated. Furthermore, in the progeny tests of resistance, the conidial suspensions of race 3 strains were spray inoculated and the inoculation was repeated one more time on each seedling after ten days to confirm their susceptibility response.

Three isolates of *A. alternata* Japanese pear pathotype, isolated from the susceptible Japanese pear cv. 'Kinchaku', and three other isolates kindly supplied



Fig. 1 Mature fruit of pear cv. Yutaka at harvest time in late September (Saitama Prefecture, Japan)

Table 1 Outline of inoculation experiments using potted trees

Time	Disease	Pear cultivar or strain	Inoculum source
June 2009	Scab	Yutaka and Kousui	Conidia (races 1, 2 and 3) from a cellophane culture
February 2010	Scab	Yutaka and Kousui	Conidia (race 1) from naturally occurring lesions
April 2010	Scab	Yutaka and Kousui	Conidia (race 1) from naturally occurring lesions
April to June 2012	Scab	Yutaka, Kousui and Mamenashi 12	Conidia (race 1) from a cellophane culture
April 2013	Scab	Progeny seedlings, Yutaka, Natsushizuku, Kousui and Mamenashi 12	Conidia (race 3) from naturally occurring lesions
February 2009	Black spot	Yutaka and Nansui	Conidia from a culture
May 2010	Black spot	Yutaka and Nansui	Conidia from a culture
May 2011	Black spot	Yutaka and Nansui	Conidia from a culture
April 2012	Black spot	Yutaka and Nansui	Conidia from a culture
April 2012	Anthraxnose	Yutaka, Niitaka and Kousui	Conidia from a culture

by F Yasuda, Tottori Prefectural Horticultural Experiment Center, were employed. Conidial suspensions ($1-5 \times 10^5$ conidia mL^{-1} distilled water (DW)) were prepared separately and mixed prior to inoculation. Cvs Yutaka and Nansui (the latter highly susceptible to black spot disease), three replicate plants of each, were used. Young actively growing shoots of potted trees were spray inoculated, whereafter the trees were kept in a moist chamber at 25 °C for 24 h, followed by transferring to a phytotron maintained at 25 °C. Two days after inoculation, the development of black spot symptoms was recorded using the following scale: 0 = no visible symptoms, 1 = < 25, 2 = < 50, 3 = < 75, 4 = > 76% of the leaf area diseased. Disease severity was calculated as follows: $([4A + 3B + 2C + 1D]/4M) \times 100$, where *A*, *B*, *C*, and *D* is the number of leaves corresponding to the



Fig. 2 Seedlings of progeny from the cross of scab susceptible cv. Natsushizuku with cv. Yutaka used for an inoculation test with *Venturia nashicola*

scale 4, 3, 2, and 1, respectively, and *M* is the total number of leaves assessed. As the incubation period of the black spot fungus is short, detached young leaves, three for each cultivar, were also prepared from the potted trees of cvs Yutaka, Nansui, Kousui and Hongli. The lower surface of the leaves were spray inoculated with conidial suspensions of *A. alternata* and placed in a covered plastic container at 25 °C in the darkness for three days to maintain high humidity. The same number of uninoculated detached leaves were employed as a control. The tests were conducted two to four times, depending on the pear cultivars. The *A. alternata* Japanese pear pathotype is well known to produce the host-specific (= host-selective) AK-toxin, a primary determinant of its pathogenicity (Nishimura and Kohmoto 1983; Otani 2000). Therefore, a culture filtrate of the pathogen isolates containing the AK-toxin was prepared after incubation at 25 °C in modified Richards liquid medium (10 g KNO_3 , 5 g KH_2PO_4 , 2.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 g sucrose, 0.02 g FeCl_2 , 1 L DW) for about four weeks. Using a micropipette, aliquots (10 μl) of the culture filtrate were placed on the left hand side of the upper surface of detached young leaves, three for each of cvs. Yutaka, Nansui and Kousui. Same amounts of DW was placed on the right hand side of the same leaves as a control. Prior to the treatment, three small superficial wounds of approximately 0.5 mm in diameter were made with a sterile needle on each side of the leaf. The treated leaves were placed in a covered humid plastic container, kept at 25 °C in the dark for four days. Presence of necrotic lesions was examined visually one, two and three days after inoculation. Furthermore, the formation of leaf necrosis was examined visually

four days after treatment with the culture filtrate of the *A. alternata* Japanese pear pathotype.

For anthracnose, three stock isolates of *C. gloeosporioides* sensu lato from the Japanese pear cvs. Niitaka and Housui were cultured on PDA plates at 25 °C for one week in the dark, whereafter conidial suspensions were made. In April 2012, potted trees of cv. Yutaka, and cvs Niitaka (susceptible reference) and Kousui (resistant reference) were spray inoculated with conidial suspensions (approximately 5×10^5 conidia mL⁻¹ DW). Three replicate trees were employed for each cultivar. After incubation at 25 °C in a moist chamber for 48 h, the inoculated trees were maintained in a screen-house outdoor (approximately 15 to 25 °C). The experiments were replicated twice. The development of brown spot lesions was observed visually seven days after inoculation, and incidence was recorded as described above.

In the preliminary test conducted in 2009, leaves of cv. Kousui inoculated with race 1 used as a reference, were heavily infected with scab. Sporulating lesions were found on 83.3% of the leaves, and a disease severity of 68.1 was recorded. In contrast, none of the inoculated leaves of cv. Yutaka produced sporulating lesions or even symptoms of scab infections (Fig. 3). In the subsequent inoculation tests, three replicates for each cultivar were employed over time. In the tests conducted in February 2010, the incidence was 17.5 ± 15.8 and severity was 52.4 ± 17.4 on cv. Kousui, whereas no symptoms developed on cv. Yutaka. In tests performed in April 2010, the ratio of sporulating leaves and disease severity was $90.7 \pm 5.3\%$ and 76.2 ± 6.5 , respectively, on cv. Kousui, but no visible symptoms

appeared on cv. Yutaka. In the tests performed in April 2012, $45.8 \pm 7.3\%$ of cv. Kousui leaves had sporulating scab lesions, and the disease severity was 30.8 ± 3.6 on this susceptible cultivar. No scab lesions occurred on the leaves of cv. Yutaka. In the drop inoculation tests performed in June 2009, the isolates of race 1 (JS-115 and Yasato 2-1-1), race 2 (Mamenashi 12A No. 1-3 and Mamenashi 12A No.1-4) and race 3 (Mamenashi 12B No.1-1 and Mamenashi 12B No. 53-1), reported formerly (Ishii et al. 2002), were employed, but no symptoms appeared on the leaves of cv. Yutaka, irrespective of pathogen races. High resistance of cv. Yutaka to race 1, the major race of *V. nashicola*, was further confirmed in the inoculation test carried out in April through June 2012. The two isolates of race 1 produced typical sporulating symptoms on the susceptible cv. Kousui but not on cv. Yutaka or on strain Mamenashi 12 (Table 2). Seedlings obtained from the cross between cvs. Natsushizuku and Yutaka did not develop visible symptoms on 5 of 23 of the genotypes. On the remaining 18 seedlings, sporulating lesions developed. A Chi square test for scab susceptibility yielded 0.718, and the expected value for a 3:1 (susceptibility: resistance) segregation ratio at $P=0.05$ was 3.84. Incidence and severity of whole seedlings examined were 35.7 ± 10.0 and 32.2 ± 9.4 , respectively. Similar numbers for cv. Natsushizuku were $81.6 \pm 11.5\%$ and 80.3 ± 10.7 . In cv. Yutaka no symptoms developed, and the disease severity was 100 and 70.3 on cv. Kousui and strain Mamenashi 12, respectively, confirming the source used for inoculation to be race 3 of *V. nashicola* (Ishii et al. 2002).

When potted pear trees were spray inoculated with conidial suspensions of the Japanese pear pathotype of



Fig. 3 Differential susceptibility of pear cvs. Yutaka (left) and Kousui (right) to scab after inoculation of potted trees with conidial suspensions of *Venturia nashicola* race 1 isolates collected from

naturally infected and sporulating lesions on cv. Kousui. Disease development was assessed one month after inoculation

Table 2 Differential susceptibility of pear cultivars and strain

Isolate	Percentage diseased leaves (%)*		
	Yutaka	Kousui	Mamenashi 12
JS-115	0 (0)**	60.0 (9)	0 (0)
Yasato 2-1-1	0 (0)	53.3 (8)	0 (0)

Yutaka, Kousui and strain Mamenashi 12 after inoculation of potted trees with conidial suspensions of two *Venturia nashicola* race 1 isolates

*No. of sporulating portions/No. of portions inoculated \times 100

**The number in parentheses represents the number of leaves that were sporulating and that there were 3×5 leaves included for each cultivar/strain and isolate

A. alternata, typical black spot symptoms appeared on the leaves of the susceptible reference cv. Nansui two days after inoculation (Fig. 4). No symptoms developed on cv. Yutaka. In the experiment performed in 2010, incidence and severity were 89.9 ± 5.1 and 30.3 ± 13.2 , respectively, on cv. Nansui. The following two years, incidence and severity were 97.8 ± 0.4 and 51.1 ± 6.7 on cv. Nansui. No symptoms appeared on the leaves of cv. Yutaka. The high resistance of cv. Yutaka to black spot was further confirmed when detached leaves were spray inoculated with conidial suspensions (Fig. 5). Three days after inoculation, no lesions developed on cvs. Yutaka, Kousui and Hongli, the latter two used as resistant references, whereas leaves of cv. Nansui were covered with necrotic lesions two days after inoculation. Necrotic spots were not formed on cvs Yutaka and Kousui after treatment with the culture filtrate of the Japanese pear pathotype of *A. alternata*, which

contained the host-specific AK-toxin. Typical necrosis formed on leaves of cv. Nansui (data not shown).

C. gloeosporioides sensu lato caused brown spot symptoms on the leaves, petioles, clusters, and shoots of the susceptible reference cv. Niitaka. The disease incidence of the leaves was 100% on this cultivar in both replicated experiments. However, no symptoms developed on leaves or other plant parts of cvs. Yutaka (Fig. 6) and Kousui.

The interspecific hybrids of pear, selected from the cross between the two Chinese pear cultivars, *P. bretschneideri* cv. Yali and *P. ussuriensis* cv. Jingbaili, were reported to be highly resistant to scab (Zhang et al. 2012). In our study, the new pear cv. Yutaka, derived from the interspecific cross of *P. bretschneideri* cv. Hongli and the scab susceptible Japanese pear cv. Housui was first tested for the susceptibility to two major diseases, scab and black spot, caused by the fungal pathogens *V. nashicola* and *A. alternata* Japanese pear pathotype, respectively. Following artificial inoculation of potted trees, young leaves of cv. Yutaka developed no symptoms of scab or black spot, indicating this pear cultivar to be highly resistant to these important diseases. In subsequent inoculation tests, the monoconidial isolates of races 1, 2 and 3 of *V. nashicola* were employed as inoculum but none of them caused visible scab symptoms on cv. Yutaka. Ishii et al. (2002) previously found the pathological specialization of this fungus and only these three races have been detected within Japan so far. It may thus be expected that the introduction of cv. Yutaka in commercial pear orchards can reduce the population size of *V. nashicola*, reducing the need for fungicide applications to control scab and black spot. Reduction of fungicide applications may further

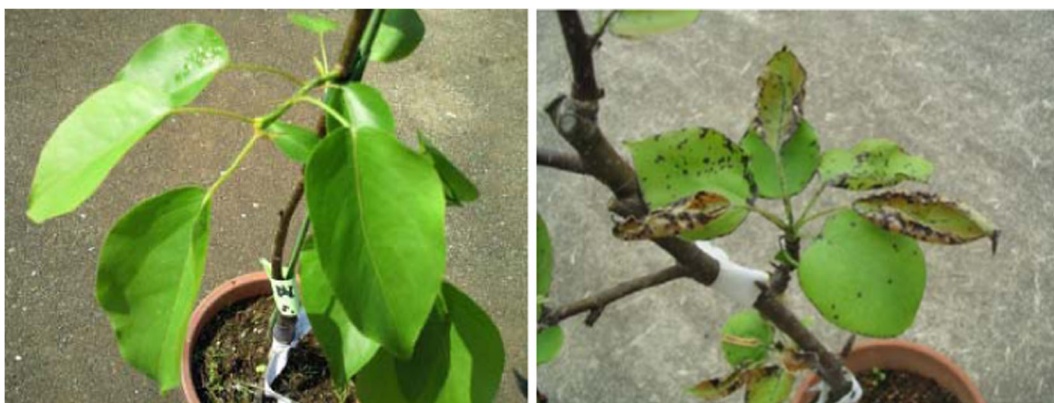


Fig. 4 Differential susceptibility of pear cvs. Yutaka (left) and Nansui (right) to black spot after inoculation of potted trees with conidial suspensions of the *Alternaria alternata* Japanese pear pathotype. Disease development was assessed two days after inoculation

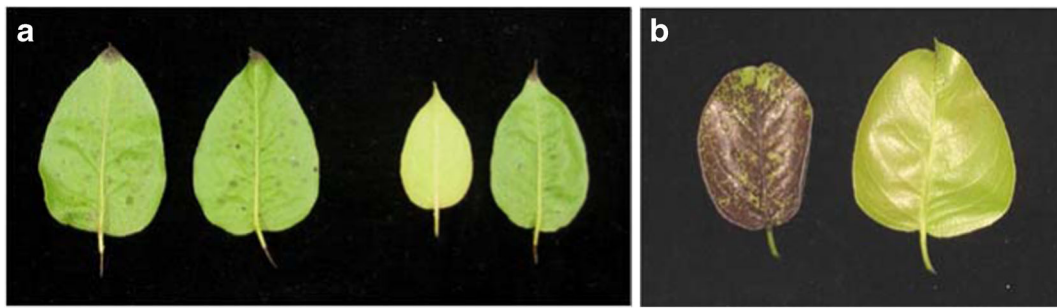


Fig. 5 Differential susceptibility of the pear cvs. Yutaka and Nansui to black spot disease after inoculation of detached leaves with conidial suspensions of the *Alternaria alternata* Japanese pear pathotype. Disease development was assessed two days after

inoculation. A, cv. Yutaka (the left two leaves were inoculated; the right two leaves, non-inoculated) and B, cv. Nansui left, inoculated; right, non-inoculated)

lower the risk of resistance development in the fungal pathogens, as part of an integrated disease management strategy in pear.

On the other hand, however, considering the potential genetic diversity of *V. nashicola* in nature, the occurrence of scab on cv. Yutaka must be followed carefully in the future. *V. inaequalis*, the cause of apple scab, has broken down resistance of the *Vf* (= *Rvi6*) gene due to the appearance of a new race of the fungus (Parisi et al. 1993; Guérin and Le Cam 2004). Moreover, the apple cultivars carrying the *Vf* gene were also damaged by the emergence of the formerly saprotrophic fungus *V. asperata*, producing an atypical scab-like symptom (Caffier et al. 2012). In European pear (*P. communis*), qualitative resistance against scab caused by *V. pirina* has been described (Pierantoni et al. 2007). In addition, the inheritance of high scab resistance in the Chinese pear cv. Hongli, one of the parents of cv. Yutaka, was examined earlier using hybrid seedlings of known

parentage (Abe and Kotobuki 1998a). The results supported the hypothesis that the high resistance phenotype of cv. Hongli was controlled by a single dominant gene, and the gene symbol *Vn* was proposed for the gene controlling this phenotype. To assume the mode of inheritance of scab resistance in cv. Yutaka, progeny tests were carried out in this study. When using 23 progeny seedlings from the cross of the scab-susceptible cv. Natsushizuku with the scab-resistant cv. Yutaka for inoculation, unexpectedly, as many as 18 seedlings exhibited clear scab symptoms accompanied with sporulation. Although only a single cross was analyzed and no information is available yet for the genetics of scab susceptibility in cv. Natsushizuku, the results indicate that the high scab resistance of cv. Yutaka is inherited in a more complex manner than dominantly or recessively in heterozygous pear.

High resistance of cv. Yutaka to black spot is obvious as no lesions or necrosis formed after inoculation with the



Fig. 6 Differential susceptibility of pear cvs. Yutaka (left) and Niitaka (right) to anthracnose after inoculation of potted trees with conidial suspensions of *Colletotrichum gloeosporioides* sensu lato. Disease development was assessed visually seven days after inoculation

Japanese pear pathotype of *A. alternata* or the treatment with the AK-toxin-containing culture filtrate of this pathogen. Despite that, future observations will also be required after planting cv. Yutaka in the field, since the pathogenic diversity of *A. alternata* based on the production of various host-specific toxins is well known (Nishimura and Kohmoto 1983; Otani 2000). A gene involved in the production of host-specific toxins is located on a small, dispensable chromosome and can be transmitted from one strain to another by horizontal transfer to enhance pathogenicity (Akagi et al. 2009). The evolution of pathogenicity in *A. alternata*, which includes seven pathogenic variants, called pathotypes, was recently reviewed (Tsuge et al. 2016). In this study, cv. Yutaka further showed resistance to anthracnose caused by *C. gloeosporioides* sensu lato. However, another species of *Colletotrichum*, *C. acutatum* sensu lato was also reported to be a pathogen of anthracnose on Japanese pear (Fukaya 2004). Both *C. gloeosporioides* and *C. acutatum* belong to species complexes (Damm et al. 2012; Weir et al. 2012; Cannon et al. 2012). Therefore, a future research subject will be to assess the susceptibility of cv. Yutaka to the species within the *C. gloeosporioides* and *C. acutatum* species complexes. On sandy pear (*P. pyrifolia*), *C. fructicola*, one of the subspecies of *C. gloeosporioides*, was characterized to cause a disease on leaves (Zhang et al. 2015). More recently, Bragança et al. (2016) reported that *C. nymphaeae* and *C. fioriniae*, the subspecies of *C. acutatum*, differed slightly in their pathogenicity and aggressiveness on fruit crops including guava and apple. Differential fungicide sensitivity has also been found between the subgroups of *C. fioriniae* (Chen et al. 2016).

In conclusions, the new interspecific pear cv. Yutaka showed high resistance to the three important diseases scab, black spot, and anthracnose, and it is the first commercial cultivar carrying high resistance to multiple serious diseases of pear in Japan.

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Compliance with ethical standards

Conflict of interest We declare that there are no conflicts of interest.

Human and animal rights This article does not contain any studies with humans or animals.

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