Varietal Differences in Susceptibility to Bacterial Spot (*Xanthomonas arboricola* pv. *pruni*) among 69 Peach Cultivars and Selections as Evaluated by Artificial Inoculation to Shoots

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Peach (*Prunus persica*) shoots were artificially inoculated with stone fruit bacterial spot bacteria (*Xanthomonas arboricola* pv. *pruni*) to evaluate varietal differences in peach genetic resources for their susceptibility to this disease. Current shoots of cultivars/selections were wounded, a bacterial suspension was injected by a syringe attached to multiple needles, and lesion length was measured a few months later. Inoculation was carried out in May, June and July with two concentrations of bacterial suspension: 10^6 cfu·mL⁻¹ or 10^8 cfu·mL⁻¹. Although the effect of inoculation time was not significant and the effect of inoculum concentration was significant, inoculation in June at a concentration of 10^8 cfu·mL⁻¹ was the most suitable treatment. Among 69 cultivars/selections tested, there was no immune cultivar, however; there were varietal differences in susceptibility to bacterial spot. ‘Nishiki’ and ‘Mochizuki’, two cultivars for canning use, ‘Chimarrita’, a Brazilian cultivar, and ‘Tsukikagami’, a table peach cultivar, were relatively resistant and may be useful sources for breeding aimed at disease resistance.

Key Words: disease resistance, genetic resources, *Prunus persica*.

Introduction

Bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* is one of the most important and serious diseases of peaches grown in areas with a lot of wind and rain. It also attacks other stone fruit crops. The symptoms of the disease are defoliation and spots on the leaves, twigs, and fruit. Leaf spots and severe defoliation damage growing trees, and spots on the fruit reduce the peaches’ commercial value. Since complete control by chemical application is difficult, the use of resistant cultivars is considered to be the most effective way to control this disease; however, immune cultivars are not known or used in Japan.

In other areas where stone fruits are grown, the susceptibility of cultivars to bacterial spot was evaluated and varietal differences were reported by Du Plessis

(1988), Keil and Fogle (1974), Martins and Raseira (1996), Medeiros et al. (2011), Randhawa and Civerolo (1985), Sherman and Lyrene (1981), and Werner et al. (1986). Similarly in Japan, orchard susceptibility of economically important cultivars has been examined several times; Yamamoto et al. (1953), Kuraoka and Kato (1955), Shiina et al. (1966), and Takanashi (1978) reported relatively resistant cultivars. However, the cultivation area of those cultivars has not increased. Additionally, breeding for disease resistance was not easy with little chance for success and the major objective of the breeding program was to improve fruit eating quality. So breeding for bacterial spot disease resistance has not been a top priority. In recent times, the commercially cultivated varieties have changed, and most currently grown cultivars have not been evaluated for their susceptibility to bacterial spot. Therefore, it is necessary to evaluate the susceptibilities of peach cultivars and selections in order to select breeding materials.

Since evaluation under field conditions is prone to be affected by climatic conditions and the density of the causal bacteria, it is advisable to evaluate trees using artificial inoculation. Topp et al. (1991) compared rating

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methods, including measurements of the number, size and incidence of leaf spots, percentage affected leaf area and stem canker spot length, and concluded that measuring the length of stem cankers at the injection sites was a simple and reproducible method. Miyake et al. (1999) made improvements to the artificial inoculation method using multiple needles on shoots and reported varietal differences in the resistance of Japanese plum (*Prunus salicina*), apricot (*Prunus armeniaca*), and a small number of peach cultivars to bacterial spot.

Bacterial spot causes spring canker, and summer canker on peach shoots. Spring canker is the main source of primary infection, and summer canker is a secondary cause (Takanashi, 1978). Since infection occurs during autumn of the previous year and the overwintered lesion becomes a spring canker, it takes a longer time from infection to measurement for spring canker than summer canker. Furthermore, spring canker may be affected by the environment. Since summer canker is easier to measure, this study focused on measuring summer canker after shoot inoculation.

Therefore, the objectives of this study were to evaluate: 1) the varietal differences in shoots for bacterial spot disease using the artificial inoculation method of Miyake et al. (1999) with multiple needles on shoots in peach genetic resources mainly consisting of peach cultivars that have been used in commercial production in Japan and selections from the NARO Institute of Fruit Tree Science (NIFTS) peach breeding program, and 2) the effects of different times of inoculation and concentrations of inoculum on the lesion length.

Materials and Methods

Plant material

Peach cultivars from the genetic resources collections and breeding selections grown as independent trees at NIFTS, Tsukuba, Ibaraki, Japan, were used. Tree ages ranged from 3 to 14 years old.

Inoculum

Xanthomonas arboricola pv. *pruni* (MAFF301420), supplied by the National Institute of Agrobiological Sciences, was the inoculant. Bacteria growing on potato dextrose agar were suspended in sterile water and adjusted to two different concentrations.

Inoculation

A syringe with ten 26-gauge needles was used to injure and inject the bacterial suspension at each site. Several current shoots, 30–40 cm long with basal diameters of about 5 mm, on trees growing in the field were artificially inoculated (Fig. 1). Three points at intervals of 7 cm per shoot, 3 shoots per treatment were lightly wounded by pricking the shoot surface with needles and injected with the bacterial suspension using multiple needles as described in the three sections below.

Fig. 1. Inoculation of shoots. Left: shoot at the time of inoculation. Right: shoot at the time of lesion length measurement.

1. Yearly effect and the genotype $\times$ year interaction for lesion length (Exp. 1)

Twenty-five peach cultivars listed in Table 1 were tested repeatedly for three years from 2006 to 2008. Current shoots were chosen in June and were subjected to multiple-needle injection of the bacterial suspension at a concentration of 10^8 cfu·mL⁻¹. Inoculated shoots were collected, lesion lengths were measured in late August or early September and the average length ($\bar{X}$) was calculated for each shoot.

Current shoots of 9 cultivars ('Mochizuki', 'Manami', 'Masahime', 'Nishiki', 'Natsuotome', 'Chiyohime', 'Shimizu Hakuto', 'Akatsuki', and 'Harrow Beauty') were wounded and injected with sterile water as the control in the same way as the artificially inoculated shoots in 2006. The average lesion length was 5.5 mm for the 9 cultivars. Therefore, the value of ($\bar{X} - 5.5$) was used as the value showing the effect of the bacterial inoculation. In addition, as the average and standard deviation were correlated, $\log_{10}(\bar{X} - 5.5)$ were used for statistical analysis (the shoot measured value). $\log_{10}(\bar{X} - 5.5)$ values of each cultivar in each year were subjected to analysis of variance (ANOVA). The model adopted here to express the measurement value is shown below:

$$P_{ijk} = \mu + gI_i + y_j + (gy)_{ij} + eI_{ijk}$$

where P_{ijk} is the shoot measured value of the i th genotype of the j th year; μ is a constant value (the overall mean); gI_i is the random effect contributed by the i th genotype; y_j is the random effect contributed by the j th year; $(gy)_{ij}$ is the interaction between the i th genotype and the j th year; eI_{ijk} is the error in the k th shoot of the i th genotype in the j th year.

Distribution of the error estimate, which was obtained as the deviation of each shoot measured value from the average shoot measured value in a cultivar and year, approached normal distribution with the Kolmogorov-Smirnov one-sample test at ($P = 0.05$).

Table 1. Peach cultivars/selections used in the present study.

Cultivar/selection	Exp. 1	Exp. 2	Exp. 3	Pedigree		Year of cultivar registration or appearing in commercial or test production ^z	Origin
				Seed parent	Pollen parent		
Table peach cultivar/selection							
Shanghai Suimitao		○				—	China
Tianjin Suimitao		○				—	China
Doyo		○			Chance seedling	1897	Okayama
Denjuro		○				1898	Kanagawa
Hakuto		○			Chance seedling	1899	Okayama
Rikaku		○		Shanghai Suimitao O.P. ^y		1900	Okayama
Tachibana Wase		○		Denjuro O.P.		1910	Kanagawa
Koyo Hakuto		○			Chance seedling	1927	Okayama
Hakuho		○		Hakuto	Tachibana Wase	1933	Kaganawa
Kiyomi		○		Kinto	Early Crawford	—	Shizuoka
Okubo		○			Chance seedling in Hakuto Orchard	—	Okayama
Shimizu Hakuto	○	○			Chance seedling in Hakuto and Okayama 3 orchard	—	Okayama
Sanekoubai		○			Local variety	—	Aomori
Nunome Wase		○			Chance seedling	1951	Aichi
Kurakata Wase		○				1951	Tokyo
Nakatsu Hakuto		○	○	Hakuto O.P.		1955	Nara
Sunago Wase		○			Chance seedling	1958	Okayama
Asama Hakuto	○	○		Koyo Hakuto bud mutation		—	Yamanashi
Akatsuki		○	○	Hakuto	Hakuho	1979	NIFTS
Kawanakajima Hakuto	○	○	○		Chance seedling	—	Nagano
Ikeda (Nagano)		○				—	Nagano
Ogonto	○	○			Chance seedling	—	Nagano
Takei Hakuho	○	○			Chance seedling in Hakuho orchard	—	Yamanashi
Reiho		○		Akatsuki bud mutation		—	Yamanashi
Hikawa Hakuho	○	○		Hakuho bud mutation		1981	Yamanashi
Benishimizu		○			Chance seedling	1983	Okayama
Saotome	○	○		Hakuho	Robin	1983	NIFTS
Kanouiwa Hakuto	○	○		Asama Hakuto bud mutation		1983	Yamanashi
Yuzora		○	○	Hakuto	Akatsuki	1983	NIFTS
Sweet Nectarine Shoko		○		Sotta Nectarine	Independence	1984	Yamanashi
Sweet Nectarine Reimei	○	○		Sotta Nectarine	Independence	1984	Yamanashi
Abe Hakuto	○	○			Chance seedling	1985	Hiroshima
Nagasawa Hakuho		○		Hakuho bud mutation		1985	Yamanashi
Gyosei	○	○		Akatsuki bud mutation		1986	Fukushima
Nishio Gold		○		Golden Peach bud mutation		1988	Okayama
Chiyohime	○	○		Koyo Hakuto	Saotome	1988	NIFTS
Chiyomaru	○	○		Momo Tsukuba 100	Nunome Wase O.P.-2	1989	NIFTS
Odoroki		○		Hakuho bud mutation		1991	Nagano
Benikunimi		○		Akatsuki	Akatsuki	1992	Fukushima
Masahime	○	○		21-18	Akatsuki	1993	NIFTS
Yoshihime	○	○		21-18	Akatsuki	1993	NIFTS
Shizuku Red		○		19-1 O.P.		1993	NIFTS
Akizora	○	○		Nishino Hakuto	Akatsuki	1995	NIFTS
Natsuki		○		Kawanakajima Hakuto	Chiyohime	1999	Nagano
Natsukko		○		Kawanakajima Hakuto	Akatsuki	2000	Nagano
Natsuotome	○	○		Akatsuki	Yoshihime	2002	NIFTS
Hatsuotome		○		Kurakata Wase	Chiyohime	2003	Fukushima
Fukuotome		○		Kurakata Wase	Chiyohime	2003	Fukushima
Hakushu		○		U-9	C2R19T182	2004	NIFTS
Himekonatsu	○	○		182-3 O.P.		2009	NIFTS
Tsukiakari	○	○		Masahime	Akatsuki	2010	NIFTS
Hinanotaki	○	○		G-62-8	G-62-8	2010	NIFTS
Tsukikagami		○		Momo Tsukuba 115	Momo Tsukuba 105	2011	NIFTS
Tsukuba 119	○	○		Momo Tsukuba 116	203-1	—	NIFTS
Tsukuba 120	○	○		Momo Tsukuba 116	203-1	—	NIFTS
Tsukuba 122		○		Sunglo	135-37	—	NIFTS
Tsukuba 124	○	○		Kawanakajima Hakuto	252-4	—	NIFTS
Tsukuba 130			○	Mochizuki	Hakushu	—	NIFTS
Canning peach							
Kanto 5		○		(Ki × T 43)	(Kohai 3 × Orange Cling 9)	1956	NIFTS
Nishiki	○	○		Kanto 12	Kanto 2	1964	NIFTS
First Gold		○		Nishiki	Hiratsuka 39	1982	NIFTS
Sweet Gold		○		Fortuna	Kanto 5	1982	NIFTS
Early Gold		○		Nishiki	Fortuna	1982	NIFTS
Mochizuki	○	○	○	Momo Tsukuba 115	139-28	2000	NIFTS
Introduced for research use from other countries to Japan							
Elberta		○		Chinese Cling O.P.		1870	U.S.A.
Taikobanto		○				—	China
Maravilha		○		Sunred	(Okinawa × Hiland) O.P.	1975	U.S.A.
Harson		○		Redskin	Sunhaven	1982	Canada
EVG-2		○				—	
Chimarrita		○		Babcock	Flordabelle	1987	Brazil

^z Listed in chronological order of year released. — indicates that the year of cultivar registration or release is uncertain, or unreleased selection.^y Open pollinated seedling.

2. Varietal differences in disease resistance to peach bacterial spot (Exp. 2)

Sixty-nine peach cultivars/selections (listed in Table 1), consisting of 57 table peach cultivars/selections, which had been grown or are presently grown commercially in Japan and selections that are being tested for future commercial production, 6 canning peach cultivars/selections and 6 peach cultivars introduced from foreign countries for research use, were tested. Every cultivar/selection was tested for two years from 2006 to 2008 in the same way as described in Exp. 1. As in Exp. 1, the value of $(X-5.5)$ was used as the value showing the effect of the bacterial inoculation, and $\log_{10}(X-5.5)$ was calculated as the shoot measured value.

3. Effect of different times of inoculation and concentrations of inoculum on lesion length (Exp. 3)

Six cultivars or selections ('Mochizuki', 'Akatsuki', 'Yuzora', 'Kawanakajima Hakuto', 'Nakatsu Hakuto', and Momo Tsukuba 130) were used. Six current shoots per cultivar/selection were chosen at three times: May, June, and July, and three current shoots per cultivar/selection and three sites per shoot were wounded by multiple-needle injections with the bacterial suspension of 10^6 cfu·mL⁻¹ or 10^8 cfu·mL⁻¹ in 2009. Inoculated shoots were collected, and the average lesion length (mm) on each shoot (X) was measured in late August. As in Exp. 1 and Exp. 2, the value of $(X-5.5)$ was used as the value showing the effect of the bacterial inoculation, and $\log_{10}(X-5.5)$ was calculated as the shoot measured value.

The three shoot measured values for each cultivar (genotype) and treatment were subjected to ANOVA. The model adopted here to express the phenotypic value is shown below:

$$P_{ijkl} = \mu + g2_i + t_j + c_k + (gt)_{ij} + (gc)_{ik} + (tc)_{jk} + (gtc)_{ijk} + e2_{ijkl}$$

where P_{ijkl} is the l th shoot measured value of the i th genotype of the j th time in the k th concentration; μ is a constant value (the overall mean); $g2_i$ is the fixed effect contributed to by the i th genotype; t_j is the fixed effect contributed to by the j th time; c_k is the fixed effect contributed to by the k th concentration, $(gt)_{ij}$ is the interaction between the i th genotype and the j th time;

$(gc)_{ik}$ is the interaction between the i th genotype and the k th concentration; $(tc)_{jk}$ is the interaction between the j th time and the k th concentration; $(gtc)_{ijk}$ is the interaction among the i th genotype, the j th time and the k th concentration; $e2_{ijkl}$ is the error in the l th shoot of the i th genotype of the j th time at the k th concentration.

Distribution of the error estimate, which was obtained as the deviation of each shoot measured value from the average shoot measured value in a cultivar, time and concentration, approached normal distribution with the Kolmogorov-Smirnov one-sample test at ($P=0.05$).

Results

1. Yearly effect and the genotype $\times$ year interaction for lesion length

The result of ANOVA showed that the effect of genotype was significant ($P < 0.01$), and the effect of year was not significant ($P > 0.05$) (Table 2). The interaction between the cultivar and the year was significant at ($P < 0.01$) (Table 2). The variance components of cultivar (σ_g^2), year (σ_y^2), the cultivar $\times$ year interaction (σ_{gy}^2), and error (σ^2), were estimated as 0.045, 0, 0.016, and 0.058, respectively (Table 3).

2. Varietal differences in disease resistance to peach bacterial spot

Bacterial spot lesions that developed on some cultivars after inoculation are shown in Figure 2. All cultivars/selections had longer lesions than the control. The lesion length data ($\log_{10}(X-5.5)$) for the 69 peach cultivars/selections artificially inoculated with bacterial suspension are presented in Figure 3. The log-transformed lesion lengths ranged from 0.476 for 'Chimarrita' to 1.606 for 'Nakatsu Hakuto'. Comparing white-fleshed cultivars/selections and yellow-fleshed cultivars/selections, there seemed to be no relationship between flesh color and lesion length. The mean lesion length value of the 57 table peach cultivars/selections in Japan was 1.090, nearly the same value as that of 'Shanghai Suimitao' (1.045). From a chronological perspective, the lesion lengths of older cultivars seemed to not differ from those of newer cultivars.

Using the error variance (σ_e^2) in Exp. 1, SE and $LSD_{0.05}$ were calculated. Each cultivar/selection value that was calculated as the average value for two years

Table 2. Analysis of variance of lesion lengths ($\log_{10}(X-5.5)$) in the artificial inoculation test to shoot with 25 cultivars/selections from 2006 to 2008 (Exp. 1)^z.

Source of variation	Sum of square	d.f.	Mean square	F-value	Expected MS
Cultivar/selection	12.301	24	0.513	8.85**	$\sigma_{e1}^2 + 3\sigma_{gy}^2 + 9\sigma_{gl}^2$
Year	0.128	2	0.064	1.10 ^{NS}	$\sigma_{e1}^2 + 3\sigma_{gy}^2 + 75\sigma_y^2$
Cultivar $\times$ year	5.052	48	0.105	1.81**	$\sigma_{e1}^2 + 3\sigma_{gy}^2$
Error	8.681	150	0.058		σ_{e1}^2
Total	26.162	224			

^{NS}, ** Nonsignificant ($P > 0.05$), or significant ($P < 0.01$), respectively.

^z X indicates lesion length for each shoot.

Table 3. Variance components estimated by the analysis of variance for lesion lengths in the artificial inoculation test to shoot with 25 cultivars/selections from 2006 to 2008 (Exp. 1).

Variance component	Estimate	Percentage of variance component to the sum of the variance components (%)
σ_{g1}^2 (cultivar)	0.045	37.8
σ_y^2 (year)	0	0.0
σ_{gy}^2 (cultivar $\times$ year)	0.016	13.4
σ_{e1}^2 (error)	0.058	48.7

Fig. 2. Artificially inoculated lesions on current shoots of some cultivars. Lesion length ($\log_{10}(X-5.5)$) is shown in parentheses.

had an error variance (σ_E^2) of $\{(\sigma_{gy}^2 + \sigma_{e1}^2/3)\}/2 = 0.018$ and SE of 0.132. $LSD_{0.05}$ was calculated as 0.367. The phenotypic variance for cultivar/selection (σ_P^2), which was the variance among the cultivar/selection values, was estimated as 0.061. The genetic variance (σ_G^2) in the whole population was estimated as $\sigma_P^2 - \sigma_E^2$, and 0.043. Broad-sense heritability, defined as σ_G^2/σ_P^2 , was 0.71. The genetic variances were estimated as 0.030, 0.090, and 0.074 for 57 Japanese table peach cultivars/selections, 6 canning cultivars, and 6 foreign cultivars, respectively.

3. Effect of different times of inoculation and concentrations of inoculum on lesion length

All the effects of the factors and their interactions were highly significant ($P < 0.01$) except for the effect of the inoculation time (Table 5). The estimates shown as κ^2 in Table 5 were used as indicators showing the extent of the effect or interaction, and the percentages of each κ^2 or the error variance σ_{e2}^2 to the sum of $7\kappa^2$ and σ_{e2}^2 were calculated (Table 6). The percentage for cultivar was the largest (44.0%), followed by that for the cultivar $\times$ time interaction (14.3%), then the inoculum concentration (13.7%), and the cultivar $\times$ concentration interaction (12.8%). The percentage was 6.8% for the cultivar $\times$ time $\times$ concentration interaction, 6.7% for the

Fig. 3. Varietal differences in log-transformed lesion length ($\log_{10}(X-5.5)$) of artificially inoculated peach shoots (2006–2008). X is the original value for lesion length. Solid and open columns indicate yellow-fleshed and white-fleshed cultivars/selections, respectively.

Table 4. Lesion length resulting from the artificial inoculation test to shoot with different inoculation times and inoculum concentrations (Exp. 3).

Cultivar/selection	Inoculum concentration	Lesion length (mm)						Average
		Inoculation time						
		May		June		July		
Momo Tsukuba 130	10 ⁶ cfu·mL ⁻¹	8.7	(0.505) ^z	10.4	(0.688)	11.8	(0.796)	10.3
	10 ⁸ cfu·mL ⁻¹	9.2	(0.573)	14.9	(0.975)	16.4	(1.039)	13.5
Akatsuki	10 ⁶ cfu·mL ⁻¹	6.4	(−0.060)	11.3	(0.763)	12.1	(0.823)	9.9
	10 ⁸ cfu·mL ⁻¹	13.3	(0.893)	15.2	(0.986)	16.4	(1.038)	15.0
Kawanakajima Hakuto	10 ⁶ cfu·mL ⁻¹	35.8	(1.482)	17.0	(1.061)	8.0	(0.395)	20.3
	10 ⁸ cfu·mL ⁻¹	44.0	(1.586)	15.9	(1.018)	14.5	(0.957)	24.8
Nakatsu Hakuto	10 ⁶ cfu·mL ⁻¹	44.2	(1.588)	40.0	(1.537)	27.7	(1.346)	37.3
	10 ⁸ cfu·mL ⁻¹	53.6	(1.682)	32.7	(1.435)	29.2	(1.375)	38.5
Mochizuki	10 ⁶ cfu·mL ⁻¹	6.0	(−0.312)	6.2	(−0.164)	5.9	(−0.428)	6.0
	10 ⁸ cfu·mL ⁻¹	9.4	(0.595)	8.7	(0.504)	12.3	(0.832)	10.1
Yuzora	10 ⁶ cfu·mL ⁻¹	8.5	(0.472)	16.4	(1.039)	16.5	(1.042)	13.8
	10 ⁸ cfu·mL ⁻¹	12.9	(0.871)	15.9	(1.018)	16.8	(1.053)	15.2
Average	10 ⁶ cfu·mL ⁻¹	18.3		16.9		13.7		16.3
	10 ⁸ cfu·mL ⁻¹	23.8		17.2		17.6		19.5

^z Average lesion length of three shoots. Number in parentheses indicate log-transformed value ($\log_{10}(X-5.5)$).

X indicates lesion length for each shoot.

Table 5. Analysis of variance for lesion length ($\log_{10}(X-5.5)$) resulting from the artificial inoculation test to shoot under different inoculation conditions; inoculation time and inoculum concentration (Exp. 3)^z.

Source of variation	Sum of square	d.f.	Mean square	<i>F</i> value	Expected MS
Cultivar/Selection	17.550	5	3.510	119.26**	$\sigma_{e2}^2 + 18\kappa_{g2}^2$
Inoculum concentration	3.275	1	3.275	111.26**	$\sigma_{e2}^2 + 54\kappa_c^2$
Inoculation time	0.139	2	0.069	2.35 ^{NS}	$\sigma_{e2}^2 + 36\kappa_t^2$
Cultivar × concentration	2.671	5	0.534	18.15**	$\sigma_{e2}^2 + 9\kappa_{gc}^2$
Cultivar × time	4.071	10	0.407	13.83**	$\sigma_{e2}^2 + 6\kappa_{gt}^2$
Time × concentration	0.303	2	0.152	5.15**	$\sigma_{e2}^2 + 18\kappa_{tc}^2$
Cultivar × concentration × time	1.188	10	0.119	4.04**	$\sigma_{e2}^2 + 3\kappa_{gct}^2$
Error	2.119	72	0.029		σ_{e2}^2
Total	31.315	107			

^{NS}, ** Nonsignificant ($P > 0.05$), or significant ($P < 0.01$), respectively.

^z *X* indicates lesion length for each shoot.

Table 6. Variance components estimated by the analysis of variance for lesion lengths in the artificial inoculation test to shoot under different inoculation conditions; inoculation time and inoculum concentration (Exp. 3).

Variance component	Estimate	Percentage of each variance component to the sum of the variance components (%)
κ_{g2}^2 (cultivar)	0.193	44.0
κ_c^2 (inoculum concentration)	0.060	13.7
κ_t^2 (inoculation time)	0.001	0.3
κ_{gc}^2 (cultivar × conc.)	0.056	12.8
κ_{gt}^2 (cultivar × time)	0.063	14.3
κ_{tc}^2 (time × conc.)	0.007	1.5
κ_{gtc}^2 (cultivar × time × conc.)	0.030	6.8
σ_{e2}^2 (error)	0.029	6.7

error, and 0.3% for the inoculation time.

While the lesion length of ‘Kawanakajima Hakuto’ and ‘Nakatsu Hakuto’ was the largest in May and the smallest in July, that of ‘Akatsuki’ and ‘Yuzora’ was the largest in July and the smallest in May (Table 4). The cultivar × time interaction was significant ($P < 0.01$) (Table 5).

Average lesion lengths were 16.3 mm and 19.5 mm for inocula of 10⁶ cfu·mL⁻¹ and 10⁸ cfu·mL⁻¹, respectively (Table 4), and the effect of the inoculum concentration was highly significant (Table 5). The effect was significant, meaning that cultivar performance shifted in parallel depending on the inoculum concentration.

Discussion

Inoculation methods to evaluate susceptibility

The result of ANOVA for cultivar/selection and year showed that the effect of the genotype was significant

and the effect of the year was not (Exp. 1). No significance of the effect of the year and little year effect in Exp. 1 mean that yearly environmental conditions have little effect on lesion lengths. The condition of peach trees may be stable, irrespective of the year tested. The data from different test years can be directly combined and compared.

Different inoculation times and inoculum concentrations were tried to determine suitable conditions for conducting the inoculation test. Current shoots of suitable size (30–40 cm long, and 5 mm basal diameter) for artificial inoculation were not available in sufficient quantity at NIFTS in May and July. There were shorter shoots in May and longer and thicker shoots in July. Since it was easier to obtain shoots in June than in May and July, June is recommended as the best time to inoculate shoots at this location. Although the effect of time was not significant, cultivar $\times$ time interaction was significant ($P < 0.01$). This result may be contributed to by ‘Kawanakajima Hakuto’, whose lesion lengths were notably larger in May than in June and July.

Varietal differences for susceptibility

Among the 69 cultivars/selections tested, there was no completely immune cultivar; however, there were varietal differences in susceptibility to bacterial spot. Peach breeding has been carried out with an emphasis on fruit quality within a small gene pool derived from ‘Shanghai Suimitao’ in Japan (Yamamoto et al., 2003). Japanese table peach cultivars/selections had small genetic variance in their resistance to bacterial spot. There were only 2 cultivars with significantly lower values (‘Benishimizu’ and ‘Tsukikagami’) and only 6 cultivars with significantly higher values (‘Nakatsu Hakuto’, ‘Kiyomi’, ‘Asama Hakuto’, ‘Kurakatawase’, ‘Sweet Nectarine Reimei’, and ‘Shizuku Red’) than the value of ‘Shanghai Suimitao’, respectively, based on the LSD. On the other hand, ‘Chimarrita’ and ‘Harson’ from the 6 foreign cultivars and ‘Michizuki’ and ‘Nishiki’ from the 6 canning cultivars/selections had significantly lower values.

Cultivars developed in areas where bacterial spot is a serious problem generally show more resistance than other cultivars selected in regions with less frequent occurrence of the disease (Keil and Fogle, 1974; Topp and Sherman, 1995; Werner et al., 1986). Therefore, cultivars that had been developed in areas prone to bacterial spot were compared with those developed in areas with infrequent occurrence of bacterial spot. In Japan, Kanagawa, Aichi, and Nara Prefectures have sustained the most serious damage from bacterial spot, whereas Yamanashi, Fukushima, and Okayama Prefectures have rarely reported the occurrence of the disease (Takanashi, 1980). The cultivars selected in Kanagawa, Aichi, and Nara, including ‘Denjuro’, ‘Tachibana Wase’, ‘Hakuho’, ‘Nunome Wase’, and ‘Nakatsu Hakuto’, had a mean value of 1.197, whereas the 21 cultivars released

from Yamanashi, Fukushima, and Okayama, including ‘Doyo’, ‘Hakuto’, ‘Rikaku’, ‘Koyo Hakuto’, ‘Ookubo’, and ‘Shimizu Hakuto’, had a mean value of 1.069 (Table 1). The difference was very small, and the relationship between the original area of the cultivar and resistance was not clear. In addition, susceptibility did not seem to change chronologically (Fig. 3), suggesting the lack of bacterial spot resistance selection in peach breeding in Japan.

The tested cultivars/selections included 4 siblings: ‘Sweet Nectarine Reimei’ (1.424) and ‘Sweet Nectarine Shoko’ (1.006), ‘Masahime’ (0.771) and ‘Yoshihime’ (1.235), ‘Hatsuotome’ (0.993) and ‘Fukuotome’ (1.020), ‘Tsukuba 119’ (1.194) and ‘Tsukuba 120’ (1.172). Lesion lengths were similar for two sibling pairs, ‘Hatsuotome’ and ‘Fukuotome’, ‘Tsukuba 119’, and ‘Tsukuba 120’, but were not similar in the other sibling pairs. The inheritance of resistance to bacterial spot should be elucidated by a crossing experiment.

From this study, ‘Chimarrita’ (0.476), a Brazilian low-chilling requirement cultivar, ‘Harson’ (0.504), a Canadian cultivar, ‘Mochizuki’ (0.514), and ‘Nishiki’ (0.522) had low values for lesion length and were selected as relatively resistant. ‘Chimarrita’ does not have adequate fruit quality for production in Japanese climates. ‘Nishiki’ and ‘Mochizuki’ are canning peach cultivars (Kajiura et al., 1966; Yamaguchi et al., 2001) and have non-melting flesh, unlike most table peach cultivars in Japan.

The inheritance of bacterial spot resistance should be investigated. If we assume quantitative inheritance and proceed to the first step of breeding based on phenotypic values, non-table peach cultivars, such as ‘Nishiki’ and ‘Mochizuki’, two canning peaches, and ‘Harson’ and ‘Chimarrita’, foreign cultivars, should be cross-parent candidates for the initial crosses. In addition, Japanese table peach cultivars/selections with high eating quality from the peach breeding program should be used as cross-parents with the aim of combining the resistance to bacterial spot with fruit quality. Notably, ‘Tsukikagami’, a table peach cultivar, was relatively resistant and may be useful genetic material for breeding.

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