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Graft incompatibility in *Prunus* spp. preceded by SPAD index reduction

Incompatibilidade de enxertia em *Prunus* spp. precedida pela redução do índice SPAD

Tainá Rodrigues das Neves[1]; Newton Alex Mayer[2*]; Bernardo Ueno[2]

Abstract

This study aimed to measure the leaf chlorophyll index (SPAD 502 Plus) in three peach cultivars grafted on different clonal rootstocks, totaling 68 scion/rootstock combinations, between five and nine months after planting in the field to determine possible graft incompatibility symptoms in *Prunus* spp. Three field trials with peach scion cultivars 'BRS-Kampai', 'Jade', and 'Maciel' grafted on 18, 25, and 25 clonal rootstocks, respectively, were established in July and August 2014. Own-rooted scion trees (without rootstock) were used as controls in each trial and all rootstocks were propagated from herbaceous cuttings. The experimental design was a randomized block with four replications and each plot consisting of a single plant. Three SPAD measurements were taken between January and April 2015. SPAD index values for rootstocks 'Mirabolano 29C' (*P. cerasifera*) and 'Marianna 2624' (*P. cerasifera* x *P. munsoniana*) were significantly lower in the three scion cultivars, culminating with tree death from graft incompatibility. Rootstocks 'Mirabolano 29C' and 'Marianna 2624' showed translocated graft incompatibility with scion cultivars BRS-Kampai, Jade, and Maciel and tree death was preceded by a reduction in SPAD index values five months after field planting. No reduction in SPAD index values was observed in the other scion/rootstock combinations nine months after planting, but these combinations should be monitored for a longer period to confirm their graft compatibility.
Key words: Chlorophyll meter. Stone fruit trees. Rootstock. Rosaceae.

Resumo

O objetivo deste trabalho foi mensurar o índice de clorofila (SPAD 502 Plus) em folhas de três cultivares de pessegueiros enxertados em diferentes porta-enxertos clonais, totalizando 68 combinações copa/porta-enxerto, entre cinco e nove meses após o plantio no campo, para qualificar os possíveis sintomas que antecedem a incompatibilidade de enxertia em *Prunus* spp. Três unidades de observação (UO) com as cultivares copa 'BRS-Kampai', 'Jade' e 'Maciel' foram estabelecidas sobre 18, 25 e 25 porta-enxertos clonais, respectivamente, no inverno de 2014. Como testemunhas, utilizaram-se as respectivas cultivares-copa autoenraizadas (sem porta-enxerto) que, assim como os porta-enxertos, também foram propagadas por estacas herbáceas. Adotou-se o delineamento experimental em blocos ao acaso com quatro repetições, sendo cada parcela composta por uma planta. O índice SPAD foi determinado em três avaliações, entre janeiro e abril de 2015. Os porta-enxertos 'Mirabolano 29C' (*P.cerasifera*) e 'Marianna 2624' (*P.cerasifera* x *P.munsoniana*) reduziram os índices SPAD nas avaliações realizadas nas três cultivares copa de pessegueiro, que culminaram com a morte das plantas por incompatibilidade. Conclui-se que os porta-enxertos 'Mirabolano 29C' (*P.cerasifera*) e 'Marianna 2624' (*P.cerasifera* x

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P.munsoniana) apresentam incompatibilidade de enxertia do tipo "translocada" com as cultivares de pessegueiro BRS-Kampai, Jade e Maciel e a morte dessas plantas foi precedida pela redução dos índices SPAD nas folhas após cinco meses do plantio. As demais combinações copa/porta-enxerto testadas não reduziram os índices SPAD até os nove meses de idade, mas necessitam ser avaliadas por maior período de tempo para se afirmar sobre a compatibilidade de enxertia.
Palavras-chave: Clorofilômetro. Frutíferas de caroço. Porta-enxerto. Rosaceae.

Introduction

Stone fruit (*Prunus* spp.) nursery trees are usually produced through the combination of two genetically distinct parts, the scion and the rootstock, to produce a composite plant that takes on the desirable features of both genotypes and control important traits including fruit size and quality and the ability to resist soil adverse conditions. However, grafting is not always successful. One cause of graft failure is graft incompatibility, the failure to form a successful and stable graft union between the rootstock and scion genotypes. A common form of incompatibility is "translocated" incompatibility, in which symptoms occur during plant development, and include early termination of scion and root growth, shriveling and early leaf drop, reduced carbohydrate translocation between scion, and rootstock leaf chlorosis evolving to leaf reddening. Thus, the appropriate choice of scion/rootstock combination is key to achieving graft compatibility (MOSSE, 1962; HARTMANN et al., 2002; GOLDSCHMIDT, 2014).

Graft incompatibility is a very complex phenomenon involving various anatomical, physiological, biochemical, and molecular interactions (PEREIRA et al., 2014) that interact with the environment (MORENO et al., 1993; ZARROUK et al., 2006a), and thus must be evaluated for each scion/rootstock combination. The SPAD (Soil Plant Analysis Diagnostic) chlorophyll meter is a portable, user-friendly, effective, and nondestructive device that can be used to estimate the nitrogen status of plants (RUBIO-COVARRUBIAS et al., 2009; DIAS et al., 2012) and the chlorophyll content in leaves. The SPAD index has been used since the 1990s in studies on the quantification of leaf chlorophyll (AMARANTE et al., 2008; JESUS; MARENCO, 2008), plant nutrition (VALE; PRADO, 2009; SIMÕES et al., 2010; PINOCHET, 2010; SANTOS et al., 2011; DIAS et al., 2012; NAVA; CIOTTA, 2013; RAMOS et al., 2013), yield estimates (GIL et al., 2002), evaluation of scion/rootstock combinations (ZARROUK et al., 2006a; PICOLLOTO et al., 2012), and translocated graft incompatibility, because low SPAD index values may be associated with the blockage of carbohydrate assimilation and nitrogen uptake (ZARROUK et al., 2006a).

Embrapa Temperate Agriculture, a unit of Embrapa, the Brazilian Agricultural Research Corporation is leading a large research project with *Prunus* spp. clonal rootstocks, testing 230 different scion/rootstock combinations under different soil and climate conditions in Brazil (MAYER et al., 2015). In the peach region of Pelotas, state of Rio Grande do Sul (RS), southern Brazil, 68 scion/rootstock combinations are being tested, many for the first time and some taxonomically distant of *P. persica*. Thus, graft incompatibility risks must be considered, and simple and practical methods that help in identifying and quantifying incompatible combinations are useful, especially in the early research stages.

Thus, in the current study, we tested the hypothesis that SPAD index values have predictive power in predicting incompatible scion/rootstock combinations in the first year after field planting. The chlorophyll content index (SPAD 502 Plus) was measured in leaves of three peach cultivars grafted on different clonal rootstocks, totaling 68 combinations, between five and nine months after field planting, to determine the possible symptoms preceding graft incompatibility in *Prunus* spp.

Materials and Methods

Field trials (FT) of scion cultivars BRS-Kampai, Jade, and Maciel grafted on clonal peach rootstocks were established at three sites in the peach region of Pelotas, RS, Brazil. Soil samples were collected at each site and analysis results (Table 1, SBCS/CQFS, 2004) helped develop acidity correction and pre-plant fertilization protocols. Nursery trees were planted in each site in July and August 2014. Each scion cultivar comprised one field trial, whose information is shown in Table 2. Weather data recorded monthly between August 2014 and April 2015 at an automated weather station located at Embrapa Temperate Agriculture (Rodovia BR-392, Km 78, 9º Distrito, Monte Bonito, Pelotas, RS, Brazil) are shown in Table 3. The weather station is 1.3, 20.3, and 26.2 km from FT 1, FT 2, and FT 3, respectively, in a straight line.

Nursery trees were grown in perforated plastic bags (30 × 18 cm) containing commercial pine bark and peat-based substrate. Clonal rootstocks of these nursery trees were produced by rooting, in an intermittent mist chamber (MAYER et al., 2013), of herbaceous cuttings from mother trees whose genotypes, selections, or cultivars in the genus *Prunus* were deposited in the "*Prunus* Rootstock Collection" at Embrapa Temperate Agriculture. The identification of rootstocks, scion cultivars and respective FTs is described in Table 4. Stem diameter was measured 5 cm above and below the grafting point immediately after planting using a digital caliper to characterize the early development of nursery trees (Table 4). Own-rooted scion cultivars (without rootstock) were used as controls in each FT. Thus, FT 1 (scion cultivar BRS-Kampai) comprised 18 rootstocks plus own-rooted controls and FT 2 (scion cultivar 'Jade') and FT 3 (scion cultivar 'Maciel') comprised 25 rootstocks plus own-rooted controls.

Three SPAD measurements of each FT were conducted between January and April 2015 (for dates and days after planting [DAP] see Tables 5 and 6). Measurements were taken using a SPAD 502 Plus portable chlorophyll meter (Konica Minolta Optics Inc., Osaka, Japan). The device has an accuracy of ± 1.0 SPAD unit, a measuring area of 2 x 3 mm, a range of 0.0–50.0 SPAD units, and determines the relative amount of chlorophyll present in leaves by measuring the absorbance of the leaf in two wavelength regions (MINOLTA, 1989). On each sampling date, SPAD index measurements were taken from the adaxial surface, at about half of the length from the apex, at 2 mm from the leaf margin in 10 healthy leaves per plant. Leaves were randomly selected around the middle third of each plant at the middle portion of branches. For each FT and in each evaluation date, measurements were taken in a single sunny morning between 09:00 and 11:30 am to minimize variations in solar radiation.

The experimental design in each FT was a randomized block of four replications with rootstocks and respective controls as treatments (Table 4), and each plot consisting of a single plant. Data were analyzed by analysis of variance (ANOVA) using the F test and means were compared by the Scott-Knott test at $p < 0.05$. All analyses were performed using SASM-Agri software (CANTERI et al., 2001).

Table 1. Results and interpretation of soil analysis from soil samples collected[x] at three sites prior to base fertilization, acidity correction, and establishment of field trials (FTs). Embrapa Temperate Agriculture, Pelotas, RS, Brazil, May 2014.

FT/scion	pH_{water} 1:1	Clay (%)	OM (%)	P	K	Na	Ca	Mg	Al	H+Al	K	Saturation (%)		$CEC_{pH\ 7}$
				mg dm^{-3}			$cmol_c\ dm^{-3}$					Al	Bases	
1. BRS-Kampai	6.5 (H)	13	1.3 (L)	2.2 (Vl)	28 (L)	9	2.2 (M)	0.9 (M)	0.0	1.6	0.1	0.0 (Vl)	67 (M)	4.8 (L)
2. Jade [y]	5.3 (L)	16	2.1 (L)	7.0 (Vl)	106 (Vh)	100	2.4 (M)	0.4 (B)	0.3	4.3	0.3	8.3 (L)	45 (L)	7.9 (M)
3. Maciel	6.1 (H)	18	2.1 (L)	41.2 (H)	101 (Vh)	16	4.2 (H)	1.3 (H)	0.0	1.0	0.3	0.0 (Vl)	85 (H)	6.9 (M)

[x] Soil depth: 0–20 cm; date of sampling: FT 1 = 04/15/2013; FT 2 = 03/21/2014; FT 3 = 02/28/2014. [y] Average from two composite samples. Interpretation of chemical analyses (SBCS/CQFS, 2004): Vl = very low; L = low; M = medium; H = high; and Vh = very high.

Table 2. Site location and characteristics of the three field trials (FTs) established with different clonal peach rootstocks. Embrapa Temperate Agriculture, Pelotas, RS, Brazil, August 2014.

Field trial	Location	Scion cultivar	Plant spacing (m)	Planting date	Elevation (m)	Slope direction
FT 1	Embrapa Temperate Agriculture, Rural Area of Pelotas, RS, Brazil (9^{th} district)	BRS-Kampai	6.0 x 3.0	08/21/2014	45–48	NE
FT 2	Rural Area of Pelotas, RS, Brazil (Santa Áurea, 7^{th} district)	Jade	5.5 x 3.0	08/14/2014	205–208	N
FT 3	Rural Area of Pelotas, RS, Brazil (São Manoel, 8^{th} district)	Maciel	5.0 x 2.6	07/29/2014	240–243	NW

Table 3. Weather data for the study period recorded at the Embrapa Temperate Agriculture weather station (31°40'59.1" S and 52°26'10.39" W; elevation 57 m). Embrapa Temperate Agriculture, 2016.

Month/year	Air temperature (°C)		Mean soil temperature (°C) at 5 cm	Total precipitation (mm)	Relative humidity (%)	Mean solar radiation ($cal.cm^{-2}.day^{-1}$)
	$Max_{aver.}$	$Min_{aver.}$				
Aug/2014	20.1	10.1	14.8	82.0	82.3	279.4
Sept/2014	20.9	12.8	17.1	179.8	86.8	287.9
Oct/2014	24.2	15.9	21.3	213.8	85.1	381.6
Nov/2014	27.0	16.7	25.2	91.9	78.2	524.1
Dec/2014	27.4	18.6	25.1	148.7	80.9	495.6
Jan/2015	29.1	20.0	26.9	168.2	81.6	520.3
Feb/2015	28.0	19.9	25.7	107.4	84.9	446.1
Mar/2015	27.7	18.2	25.7	59.7	82.9	408.5
Apr/2015	25.8	15.3	20.6	33.5	79.1	355.3

Table 4. Identification of *Prunus* spp. genotypes, selections, or cultivars used as clonal rootstocks in the three field trials (FTs) and stem diameter 5 cm above and below the grafting point at planting. Embrapa Temperate Agriculture, Pelotas, RS, Brazil, August 2014.

Rootstock	Species	Stem diameter (mm) in relation to grafting point at planting					
		FT 1: BRS-Kampai		FT 2: Jade		FT 3: Maciel	
		above	below	above	below	above	below
Barrier	*P. persica* x *P. davidiana*	4.96 a	7.71 a	3.93 c	7.60 c	5.03 c	7.92 a
Cadaman	*P. persica* x *P. davidiana*	4.57 a	6.86 b	3.89 c	6.91 c	4.36 c	6.55 b
GF 677	*P. persica* x *P. amygdalus*	-	-	4.79 b	8.64 b	4.11 d	8.19 a
G x N.9	*P. persica* x *P. dulcis*	4.4 a	8.35 a	3.73 c	7.26 c	4.36 c	8.3 a
Capdeboscq	*P. persica*	4.88 a	6.17 b	4.01 c	6.45 c	3.94 d	5.8 b
Mirabolano 29C	*P. cerasifera*	4.79 a	7.64 a	4.99 b	8.08 b	5.61 b	8.37 a
Marianna 2624	*P. cerasifera* x *P. munsoniana*	4.65 a	9.13 a	4.46 c	8.59 b	5.2 c	8.84 a
Genovesa	*P. salicina*	4.7 a	8.48 a	5.02 b	8.74 b	5.11 c	8.11 a
Rigitano	*P. mume*	4.6 a	7.22 b	3.27 c	8.60 b	4.9 c	7.26 b
Clone 15	*P. mume*	5.13 a	5.81 b	4.96 b	7.19 c	4.71 c	6.42 b
México F1	*P. persica*	-	-	3.18 c	5.24 c	3.8 d	6.13 b
I-67-52-4	*P. persica*	4.29 a	6.5 b	3.56 c	6.04 c	2.86 d	5.54 b
Tsukuba-1	*P. persica*	4.88 a	7.93 a	4.76 b	7.22 c	4.6 c	6.81 b
Tsukuba-2	*P. persica*	4.37 a	5.83 b	3.27 c	6.11 c	4.02 d	7.22 b
Tsukuba-3	*P. persica*	4.49 a	7.63 a	5.43 b	8.91 b	4.55 c	8.18 a
Okinawa	*P. persica*	4.36 a	7.06 b	4.84 b	7.27 c	4.13 d	6.52 b
Flordaguard	'Chico 11' x *P. davidiana*	5.24 a	6.49 b	4.35 c	6.58 c	5.22 c	6.62 b
Nemared	*P. persica*	3.45 a	6.3 b	3.61 c	6.04 c	4.37 c	6.28 b
Ishtara	(*P. cerasifera* x *P. salicina*) x (*P. cerasifera* x *P. persica*)	4.39 a	6.95 b	4.76 b	8.67 b	4.56 c	7.91 a
Aldrighi	*P. persica*	-	-	3.89 c	6.87 c	3.88 d	6.08 b
Tardio 01	*P. persica*	-	-	4.19 c	7.38 c	4.72 c	6.98 b
De Guia	*P. persica*	-	-	4.44 c	7.0 c	5.05 c	6.33 b
Rosaflor	*P. persica*	-	-	4.33 c	6.7 c	4.72 c	6.17 b
P. mandschurica	*P. mandschurica*	-	-	4.62 b	7.06 c	4.66 c	7.05 b
Santa Rosa	*P. salicina*	4.42 a	7.54 a	5.58 b	9.9 a	6.09 b	9.37 a
Own-rooted[(x)]	*P. persica*	5.33 a	6.71 b	7.76 a	10.07 a	7.30 a	9.33 a
$F_{rootstock}$		1.28 ^{NS}	2.65**	6.17**	6.17**	4.76**	5.24**
F_{blocks}		0.47 ^{NS}	2.5 ^{NS}	3.23*	0.11 ^{NS}	2.12 ^{NS}	2.02 ^{NS}
CV (%)		16.11	15.68	17.05	13.0	16.36	13.23

(-) indicates that the scion/rootstock combination was not included in the FT. [(x)] Because of the lack of a grafting point, stem diameter was measured at 5 cm above and below the stem insertion in the original cutting. Mean values followed by different letters in a column are significantly different by the Scott-Knott test. $F_{roostock}$, F test for rootstock; F_{blocks}, F test for blocks; CV%, coefficient of variation. ^{NS} non-significant, ** $p < 0.01$, and * $p < 0.05$.

Results and Discussion

SPAD index values are shown in Table 5 (scion cultivar BRS-Kampai) and Table 6 (scion cultivars Jade and Maciel). We measured significantly lower SPAD index values in 'Marianna 2624' and 'Mirabolano 29C' than in the other rootstocks, either in combination with BRS-Kampai (Table 5), or with cultivar Jade or Maciel (Table 6), in all evaluations. All scion combinations displayed similar SPAD index values (Tables 5 and 6). In addition, SPAD values for all the other rootstocks across scion cultivars were not significantly different from SPAD values of own-rooted controls (Tables 5 and 6). These findings differ from results by Picolloto et al. (2012), who found very low SPAD index values for cultivar 'Maciel' grafted onto eight different rootstocks, with reference rootstocks 'Okinawa clone 12', 'Capdeboscq', and 'Aldrighi' displaying significantly lower SPAD index values (27.8, 29.6, and 29.4, respectively), than rootstocks 'Okinawa clone 1', 'Rubira', 'Nemaguard', and 'Tsukuba' (32.5, 32.3, 32.3, and 32.6, respectively). Moreno et al. (1993) reported that the early reduction in chlorophyll content and chlorophyll/carotenoid ratio at the start of summer in the first year after planting preceded the occurrence of graft incompatibility symptoms in 'Fuzalode' and 'Summergrand' peach trees grafted on 10 myrobalan × myrobalan clones (*P. cerasifera*). Therefore, our data supports previous reports that there may be effects of rootstocks on the scion chlorophyll content, and that the SPAD index can be used as a quick method, in field conditions, to assist in the early detection of graft incompatibility.

Table 5. SPAD index for scion cultivar BRS-Kampai grafted on different clonal rootstocks. Embrapa Temperate Agriculture, Pelotas, RS, Brazil, May 2015.

Treatment and rootstock	Evaluation dates and days after planting (DAP) for scion cultivar BRS-Kampai		
	01/19/2015 (DAP=151)	03/02/2015 (DAP=193)	04/09/2015 (DAP=231)
T1- Barrier	38.48 a	42.38 a	43.18 a
T2- Cadaman	40.18 a	43.38 a	43.48 a
T3- G x N.9	40.15 a	41.78 a	42.65 a
T4- Capdeboscq	41.08 a	41.03 a	42.45 a
T5- Mirabolano 29C	33.68 b	28.1 b	24.13 b
T6- Marianna 2624	30.58 b	31.43 b	26.23 b
T7- Genovesa	41.48 a	44.68 a	45.43 a
T8- Rigitano	43.0 a	48.85 a	43.18 a
T9- Clone 15	42.83 a	42.45 a	45.55 a
T10- I-67-52-4	39.15 a	41.13 a	41.38 a
T11- Tsukuba-1	40.0 a	42.55 a	42.3 a
T12- Tsukuba-2	39.43 a	42.13 a	43.1 a
T13- Tsukuba-3	39.13 a	41.13 a	41.65 a
T14- Okinawa	39.55 a	41.1 a	42.85 a
T15- Flordaguard	39.5 a	40.18 a	42.85 a
T16- Nemared	40.4 a	42.33 a	41.6 a
T17- Ishtara	39.98 a	43.4 a	44.95 a
T18- Santa Rosa	41.4 a	43.13 a	43.7 a
T19- Own-rooted	43.13 a	41.38 a	42.4 a
$F_{rootstock}$	1.91*	4.61**	9.22**
F_{blocks}	1.41 ^{NS}	0.5 ^{NS}	2.97*
CV (%)	11.0	9.33	9.24

Mean values followed by different letters in a column are significantly different by the Scott-Knott test. DAP, Days After Planting. $F_{roostock}$, F test for rootstock; F_{blocks}, F test for blocks; CV%, coefficient of variation. ^{NS} non-significant, ** $p < 0.01$, and * $p < 0.05$.

Table 6. SPAD index values for scion cultivars Jade and Maciel grafted on different clonal rootstocks. Embrapa Temperate Agriculture, Pelotas, RS, Brazil, May 2015.

Treatment and rootstock	Scion cultivar Jade Evaluation dates and days after planting			Scion cultivar Maciel Evaluation dates and days after planting		
	01/20/2015 (DAP=159)	02/19/2015 (DAP=189)	04/07/2015 (DAP=236)	01/21/2015 (DAP=176)	02/23/2015 (DAP=209)	04/08/2015 (DAP=253)
T1- Barrier	43.48 a	44.53 a	45.68 a	43.83 a	43.93 a	47.45 a
T2- Cadaman	43.23 a	42.33 a	45.35 a	42.58 a	42.48 a	44.2 a
T3- GF 677	43.63 a	44.8 a	44.05 a	42.65 a	42.38 a	45.08 a
T4- G x N.9	43.03 a	45.33 a	44.05 a	42.08 a	41.8 a	45.33 a
T5- Capdeboscq	43.3 a	42.38 a	44.85 a	41.1 a	43.18 a	45.43 a
T6-Mirabolano 29C	32.53 b	31.35 b	33.4 b	25.0 b	26.03 b	-
T7- Marianna 2624	24.98 c	31.6 b	34.4 b	27.0 b	23.18 b	-
T8- Genovesa	46.65 a	46.55 a	47.1 a	44.0 a	44.73 a	43.55 a
T9- Rigitano	44.65 a	43.38 a	45.23 a	43.0 a	43.98 a	45.83 a
T10- Clone 15	43.63 a	44.98 a	45.83 a	42.78 a	43.38 a	44.53 a
T11- México F1	43.43 a	42.1 a	45.3 a	41.28 a	43.08 a	45.28 a
T12- I-67-52-4	42.83 a	41.95 a	44.6 a	41.2 a	42.08 a	44.23 a
T13- Tsukuba-1	42.28 a	44.63 a	47.43 a	38.15 a	40.3 a	45.95 a
T14- Tsukuba-2	41.55 a	43.15 a	44.45 a	40.2 a	42.3 a	45.0 a
T15- Tsukuba-3	41.48 a	44.2 a	45.18 a	40.53 a	40.83 a	43.78 a
T16- Okinawa	42.38 a	43.05 a	45.78 a	41.73 a	41.1 a	45.38 a
T17- Flordaguard	42.15 a	41.35 a	45.43 a	40.7 a	42.68 a	46.1 a
T18- Nemared	42.93 a	41.05 a	44.98 a	38.53 a	39.55 a	44.53 a
T19- Ishtara	47.0 a	44.83 a	45.95 a	43.65 a	44.53 a	46.25 a
T20- Aldrighi	43.13 a	40.98 a	43.05 a	42.25 a	42.33 a	43.9 a
T21- Tardio 01	41.7 a	42.4 a	44.43 a	41.68 a	43.53 a	46.85 a
T22- De Guia	43.4 a	41.63 a	44.88 a	42.28 a	41.53 a	45.83 a
T23- Rosaflor	43.15 a	43.03 a	45.88 a	41.38 a	42.23 a	43.0 a
T24- *P. mandschurica*	43.08 a	40.88 a	47.2 a	42.68 a	44.28 a	46.35 a
T25- Santa Rosa	43.83 a	44.5 a	45.25 a	42.35 a	41.58 a	46.53 a
T26- Own-rooted	43.9 a	42.1 a	44.78 a	41.4 a	42.4 a	45.73 a
$F_{rootstock}$	3.74**	4.32**	19.85**	13.93**	17.9**	1.5^{NS}
F_{blocks}	0.54^{NS}	1.86^{NS}	1.84^{NS}	1.17^{NS}	0.69^{NS}	4.82**
CV (%)	10.53	8.02	3.28	5.96	5.8	4.03

Mean values followed by different letters in a column are significantly different by the Scott-Knott test. DAP, Days After Planting. $F_{roostock}$, F test for rootstock; F_{blocks}, F test for blocks; CV%, coefficient of variation. ^{NS} non-significant, ** $p < 0.01$, and * $p < 0.05$. (-) dead plants.

In the second and third evaluations for the three scion cultivars, only one combination had an average SPAD index < 40.0 ('Maciel'/'Nemared' = 39.55) other than the three scion cultivars grafted on 'Marianna 2624' and 'Mirabolano 29C' rootstocks (all values < 34.4). According to Rubio-Covarrubias et al. (2009), SPAD index values of 42.2 and 40.4 correlated with total leaf N contents of 3.68% and 3.22% (i.e., above the normal recommended levels) in nectarine cv. Fantasia/'Nemaguard' trees in the second and third year after planting, respectively. According to these authors, optimal total leaf N levels and SPAD index values decrease with plant age in the first three years, at soil and climate conditions of Davis, CA, USA. However, there are no studies correlating the SPAD index with optimum levels of leaf N for peach trees grown in Southern Brazil. For grafting incompatibility studies, the SPAD index

can only support the diagnostic, as no definition of threshold values that can be used to determine if a combination scion/rootstock is compatible exists (PICOLOTTO et al., 2012).

Early symptoms of plant growth reduction and leaf blade yellowing and shriveling were observed across scion cultivars in virtually all replications grafted onto 'Mirabolano 29C' (Figures 1a, 1b, 3a, and 3b) and 'Marianna 2624' (Figures 2a, 2b, 4a, and 4b) rootstocks. Some leaves, especially in scion cultivar Jade (Figures 1a and 2a), also started to show red circular spots. These spots were also observed in scion cultivars Maciel and BRS-Kampai, but were more prominent after eight months of age. Symptoms evolved to early leaf drop at the end of March and, at the end of April 2015, all plants in the 'Jade'/'Mirabolano 29C', 'Jade'/'Marianna 2624', 'Maciel'/'Mirabolano 29C', and 'Maciel'/'Marianna 2624' combinations had died from graft incompatibility (Figures 1b, 2b, 3b, and 4b). The symptoms observed in leaves from plants grafted on 'Mirabolano 29C' and 'Marianna 2624' rootstocks are identical to those described by Moreno et al. (1993) and Zarrouk et al. (2006a), who reported translocated incompatibility in peach and nectarine cultivars grafted on 'Marianna 2624', 'Myrobalan 29C', and other myrobalan clones (*P. cerasifera*). Graft incompatibility due to increased phenol concentration and peroxidase activity had also been reported in rootstocks 'Mirabolano' and 'Mariana' grafted on peach cultivars 'Diamante' and 'Eldorado' (RODRIGUES et al., 2001).

The progression of incompatibility symptoms was slower in scion cultivar BRS-Kampai than in cultivars 'Jade' and 'Maciel', especially in some replicates from rootstocks 'Mirabolano 29C' and 'Marianna 2624'. Sixteen months after planting (December 2015), two 'BRS-Kampai'/'Marianna 2624' plants were still alive, one showing few symptoms and the other with severe incompatibility symptoms. A single 'BRS-Kampai' plant grafted on 'Mirabolano 29C' was alive, but its growth had been severely compromised due to incompatibility symptoms. Similar results were found by Moreno et al. (1993), who reported that incompatibility symptoms in the first year might be expressed in only a few replications from any scion/rootstock combination, even in clone combinations. Those authors attribute differences in manifestation of symptoms between replications to variations in plant preparation, including insufficient control of factors affecting cuttings before and after rooting, rooting and grafting technique, and the characteristics of the grafted buds. In addition, soil and climate conditions, which are difficult to replicate in the field, can affect the scion/rootstock relationship (Tables 1 and 3). Kester and Hansen (1964) reported that almond cultivars and selections grafted on 'Marianna 2624' exhibited different behaviors, from lack of symptoms up to full incompatibility, six years after field planting, and Grasselly (1983) reported that some 'Myrobalan' clones (*P. cerasifera*) showed graft compatibility with various peach cultivars. Even though early detection of graft incompatibility has been reported (RODRIGUES et al., 2001; ERREA; BORRUEY, 2004; ZARROUK et al., 2006b; ZARROUK et al., 2010), the methods usually have limitations and evaluation cycles in the field for some rootstock combinations may require several years before any assumptions on compatibility and yield efficiency can be made, especially when genotypes are taxonomically distant (GOLDSCHMIDT, 2014). Thus, graft incompatibility is one of the major hurdles for rootstock improvement and analysis (CIOBOTARI et al., 2010).

Figure 1. Translocated graft incompatibility in the 'Jade'/'Mirabolano 29C' combination showing yellowing, shriveling, and red spots in leaves (a) and similarity in stem diameter above and below the grafting point (←) at the time of death nine months after planting (b).

Figure 2. Translocated graft incompatibility in the 'Jade'/'Marianna 2624' combination showing yellowing, shriveling, and red spots in leaves (a) and similarity in stem diameter above and below the grafting point (←) at the time of death nine months after planting (b).

a

b

Figure 3. Translocated graft incompatibility in the 'Maciel'/'Mirabolano 29C' combination showing early signs of yellowing and shriveling in leaves (a) and similarity in stem diameter above and below the grafting point (←) at the time of death nine months after planting (b).

a

b

Figure 4. Translocated graft incompatibility in the 'Maciel'/'Marianna 2624' combination showing early signs of yellowing and shriveling in leaves (a) and similarity in stem diameter above and below the grafting point (←) at the time of death nine months after planting (b).

a

b

Stem diameter above and below the grafting point at nine months after planting was similar across 'Jade'/'Mirabolano 29C', 'Jade'/'Marianna 2624', 'Maciel'/'Mirabolano 29C', and 'Maciel'/'Marianna 2624' plants with virtually no visible differences. Mean stem diameter values (Ø above the grafting point /Ø below the grafting point, in mm) for these combinations were as follows: 'Jade'/'Mirabolano 29C' = 14.6/14.5; 'Jade'/'Marianna 2624' = 12.6/13.0; 'Maciel'/'Mirabolano 29C' = 14.3/11.0; 'Maciel'/'Marianna 2624' = 16.5/15.1. Figures 1b, 2b, 3b, and 4b, showing 'Jade'/'Mirabolano 29C', 'Jade'/'Marianna 2624', 'Maciel'/'Mirabolano 29C', and 'Maciel'/'Marianna 2624' plants, respectively, at nine months of age (end of April 2015), illustrate the stem diameter similarity above

and below the grafting point. Longitudinal stem cross-sections at the grafting point showed no necrotic lines, indicating that graft incompatibility was not localized, but translocated.

No differences in SPAD index values were observed among the other scion/rootstock combinations and between these and own-rooted controls. However, because graft incompatibility symptoms may occur after several years (HARTMANN et al., 2002; GOLDSCHMIDT, 2014), the evaluations should continue, especially those regarding plant yield, vigor, and nutrition, to confirm the graft compatibility of the other combinations and the potential of each rootstock. The SPAD index is a fast, convenient and non-destructive method that helps to detect incompatible scion/rootstock combinations under field conditions, as early as four to six months before the death of the plants. However, the SPAD index should be employed together with other methods for grafting incompatibility assessment, as chlorophyll content can be influenced by other factors such as inadequate nutrition of the plants.

Conclusions

Rootstocks 'Mirabolano 29C' (*P. cerasifera*) and 'Marianna 2624' (*P. cerasifera* x *P. munsoniana*) showed translocated graft incompatibility with peach cultivars BRS-Kampai, Jade, and Maciel and plant death was preceded by a reduction in SPAD index values five months after field planting.

The SPAD index can be used as a predictive method to assess graft incompatibility in *Prunus* spp., as long as it is supported by other methods .

No reduction in SPAD index values was observed in the other scion/rootstock combinations nine months after planting, but these combinations should be monitored for a longer period to confirm their graft compatibility.

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