The phenotypically quantitative nature of hypersensitivity of European plum (*Prunus domestica* L.) against the Plum pox virus and its description using the hypersensitivity index

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ABSTRACT: More than 1,300 seedlings of European plum originating from crossing combinations with at least one parent showing hypersensitivity resistance against PPV were analyzed for their reaction to artificial inoculation with PPV using the double grafting method with virus infected interstem. It was shown that the hypersensitivity resistance against the virus is a phenotypically quantitative trait. The different kinds of symptoms observed in the test system, which contribute to the hypersensitivity resistance, range from weak necrosis on the leaf blade and on the stem to the death of the whole young shoots. A hypersensitivity index was developed which helps to determine the degree of hypersensitivity resistance of an individual genotype. Its use is strongly recommended as selection tool in breeding for hypersensitivity resistance.

Keywords: sharka disease; hypersensitivity; resistance; hypersensitive response; hypersensitivity index; hypersensitivity class; resistance breeding; stone fruit

The sharka disease, first described by Atanasoff (1933), is the most devastating virus disease in temperate stone fruit production. It is caused by the Plum pox virus (PPV). Since the first description of this disease, great efforts were made to find resistance in affected fruit species such as peach, apricot, Japanese and European plum. In recent years, the hypersensitivity resistance of European plum (*Prunus domestica* L.) gained more and more attention as it is, until now, the only known resistance mechanism that can efficiently prevent the PPV infection of trees of this fruit species in the orchards. However, there are only a few detailed descriptions of the hypersensitivity of European plum against PPV (Kegler et al. 1991; Hartmann 1998). All these descriptions refer to individual genotypes or few descendants of crossing combinations, not to a broad range of progenies of breeding programs. In order to get a survey of all different kinds of phenotypically visible characteristics of different degrees of hypersensitivity against PPV, genotypes originating from different crossing combinations with at least one hypersensitive parent were investigated. The aim was to describe the range of hypersensitivity traits and to develop an applicable system that can be used to characterize the degree of hypersensitivity of a respective genotype and to compare different genotypes with respect to this character.

MATERIAL AND METHODS

Genotypes to be tested for their degree of hypersensitivity were inoculated with PPV using the double grafting method described by Kegler et al. (1994). At first, the upper scion was grafted onto the interstem, immediately after that this combination was grafted onto seedlings of *Prunus cerasifera* as rootstock using the grafting machine Topgrafter (Raggett Industries, New Zealand) for both grafting steps. As PPV inoculum, budsticks cut from *P. domestica* trees growing in the sharka experimental orchard in Weil der Stadt showing strong PPV symptoms for several years were used. Budsticks were taken from varieties known to be sensitive to PPV

and showing clear PPV symptoms on the leaves such as Katinka and Čačanska rodna. The PPV infection of the mother trees was shown by ELISA and RT-PCR tests (EPPO 2004). These trees were infected with a PPV-D isolate. The graftings were done in January, the plants were potted in 3 l plastic containers and cultivated in a heated greenhouse from February until June (minimal temperature 15°C). In some cases, the plants were cultivated for two years in order to observe their behaviour after a dormancy period. In the years 2003–2006, a total of 1,329 genotypes originating from 28 crossing combinations were tested, each of them in three replications (tree double graftings per genotype) (Table 1).

Rootstock suckers were removed continuously. The shoots of the interstem were pinched back after they had developed three fully expanded leaves. PPV symptoms could be observed on these leaves in order to ensure the infection of the individual plant. The shoots developing from the upper scion were not treated.

The response of each individual plant to PPV infection was rated three times (four, eight and ten weeks after potting). Table 2 shows the rated traits, their possible characteristics and the rating values assigned to the characteristics.

Plants were declared to be PPV infected when the leaves of the interstem showed clear PPV symptoms. Moreover, DASI-ELISA tests using PPV universal antibodies 5B-IVIA (Cambra et al. 1994; EPPO 2004) and RT-PCR tests (Wetzel et al. 1991; EPPO 2004) were used to ensure the PPV infection of individual plants. The serological and molecular biological diagnostic methods were used in all cases where no PPV symptoms and no sign of hypersensitivity could be observed on the shoots of the upper scion and, simultaneously, the leaves of the interstem showed clear PPV symptoms.

For the histological investigation of necrosis on the stem of young shoots, four segments of shoots of each rating value of stem necrosis were fixed in phosphate buffered glutaraldehyde-formaldehyde solution (Karnovsky 1965), embedded in resin [glycolmethacrylate/methacrylate method described by Ruddell (1967a,b) and modified by Hermanns and Schulz (1981)] and cut into 8 µm sections. The sections were stained using a astrablue-chrysoidine-new fuchsin (ACN) staining solution (Hermanns, Schulz 1981; Neumüller 2005).

The result of a resistance test always depends on the test method used. In order to investigate the influence of the test system on the expression of the hypersensitivity trait, 68 of the genotypes were not only tested in the greenhouse but also in the orchard.

Table 1. Parentage and number of genotypes tested. Jojo, Ort × Stan 34, Ort × Gerst 17 and Hoh 4465 are hypersensitive parents

Crossing combination	No. of genotypes tested
Hoh 4465 × Jojo	71
Hoh 4515 × Jojo	81
Čačanska najbolja × (Ort × Stan 34)	14
Čačanska rodna × (Ort × Stan 34)	32
Čačanska lepotica × (Ort × Stan 34)	16
Elena × (Ort × Stan 34)	63
Fellenberg × Jojo	21
Hanita × Jojo	52
Jojo × Haganta	62
Jojo × Hoh 1468	70
Jojo × Hoh 4465	14
Jojo × Čačanska rodna	62
Jojo × Fellenberg	76
Jojo × Felsina	91
Jojo × Hanita	49
Jojo × German Prune, clone Gunser	20
Jojo × German Prune, clone Schüfer	31
Jojo × German Prune, clone Wolff	50
Jojo × Jojo	33
Jojo × Katinka	51
Jojo × Klon 108	68
Jojo × Klon 128	43
Jojo × (Ort × Gerst 17)	45
Jojo × Presenta	77
Jojo × Zwintschers Frühe	47
(Ort × Stan 34) × Hanita	43
(Ort × Stan 34) × Jojo	47
Tegera × Hoh 6482	50
Sum	1,329

For that purpose, budwood of those genotypes was grafted onto 10-year-old plum trees that had been artificially inoculated with PPV-D in the second year of their life. Trees of varieties known to be sensitive to PPV and showing clear PPV symptoms on the leaves such as Katinka and Čačanska rodna were used. They were proved to be infected with PPV by ELISA. Some of the genotypes were grafted using the Topgrafter machine at the end of April 2003 and 2004, some of them using the bark grafting method in the middle of May of the same years. The scion wood of each genotype was grafted onto two different trees with two replications on one of them and one replication on the other one. One tree held up to 10 different genotypes. The rating was done at the

Table 2. Rating scale for hypersensitivity tests. Rated traits, their possible characteristics and the rating values assigned to the characteristics (brackets: abbreviations used in the text)

Part of the grafting	Trait	Parameter value	Rating value
Interstem	PPV symptoms on the leaves	without	0
		very few	1
		few	2
		medium	3
		heavy	4
		heaviest	5
Cultivar	necrosis on the leave blade (NLB)	without	0
		single necrotic spots near the leaf veins	1
		single necrotic spots distributed over the leaf	2
		necrotic spots	3
		large necrotic spots on the leaf which touch one another	4
		leaf completely necrotic	5
	necrosis on the stem young shoot ('bark') (NS)	without	0
		some necrotic scores	1
		necrotic spots	2
		longish necrotic strips	3
		necrotic strips covering more than one internode	4
		necrotic strips reaching from the base to the top of the shoot	5
	death of the shoot tip on the most diseased shoot (DST(d))	without	0
		commencing wilting	1
		wilting shoot tip	2
		wilting, first leaves drying up	3
		some necrotic scores at the base of the shoot still green, all other parts of the shoot dried	4
		shoot completely dried	5
	length of the shoot with the most diseased shoot tip (L(DST(d)))	longer than 20 cm	1
		12–20 cm	2
		8–12 cm	3
		3–8 cm	4
		shorter than 3 cm	5
	death of the shoot tip on the healthiest shoot (DST(h))	without	0
		commencing wilting of the shoot tip	1
		wilting shoot tip	2
		wilting shoot tip, first leaves drying up	3
		some necrotic scores at the base of the shoot still green, all other parts of the shoot dried	4
		shoot completely dried	5
	length of the shoot with the healthiest shoot tip (L(DST(h)))	longer than 20 cm	1
		12–20 cm	2
		8–12 cm	3
		3–8 cm	4
		shorter than 3 cm	5

Table 2 to be continued

Part of the grafting	Trait	Parameter value	Rating value
Cultivar	PPV symptoms on the leaves	without	0
		very few	1
		few	2
		medium	3
		heavy	4
		heaviest	5

Table 3. Classification of hypersensitivity classes based on different levels of the hypersensitivity index

Hypersensitivity index (HI)	Reaction to PPV inoculation	Hypersensitivity class (HC)
(0.00; 0.10)	normal sensitivity	0
(0.10; 0.40)	weak hypersensitivity	1
(0.40; 0.70)	moderate hypersensitivity	2
(0.70; 1.00)	strong hypersensitivity	3

Table 4. Hypersensitivity index (HI) and hypersensitivity classes (HC) of some selected plum genotypes. Inoculation with PPV via double grafting. Time of observation: five months

Genotype (parents in the case of breeding clones)	HI	HC
Čačanska rodna	0.00	0
Common prune, clone Schüfer	0.00	0
Felsina	0.00	0
Katinka	0.00	0
Hoh 5363 (Jojo × Presenta)	0.11	1
Clone 108 (K4 open pollination)	0.48	2
Clone 128 (K4 open pollination)	0.68	2
Jojo	0.71	3
Hoh 4517 (Elena × (Ort × Stan 34))	0.76	3
Hoh 4465 (Hanita × (Ort × Stan 34))	0.93	3
Hoh 6563 ((Ort × Stan 34) × Jojo)	0.98	3
Hoh 6587 ((Ort × Stan 34) × Jojo)	1.00	3

end of June and at the beginning of August following the same scheme as mentioned above (Table 2).

RESULTS

In the greenhouse test system, the buds of the scion woods started to grow about six weeks after potting. At the very beginning, no differences in the behaviour of the different genotypes were detected. After the scion shoot had reached a length of about 2 cm, the first differences between genotypes appeared. Within the three replications, all the plants showed the same type of reaction.

Death of young shoot tips

Ten days after budbreak, the shoot tips of some genotypes got olive green, sometimes as well slightly red due to the accumulation of anthocyanins. The leaves close to the shoot tip displayed wilting symptoms. Often, the shoot tip curved like an arc. Within a few days, the whole shoot tip got necrotic, finally the whole shoot died.

In some single cases, at the base of the necrotic shoots new buds developed which grew out subsequently. These new shoots died off completely, sometimes very fast, sometimes after several months, sometimes after the following dormancy period. No PPV could be detected in those new shoots using ELISA and RT-PCR-techniques as long as they grew normally and did not show symptoms of wilting and necrosis.

In a number of genotypes the symptoms of the death of young shoot tips occurred not immediately after the budbreak, but several weeks later when the young shoots had reached a length of about 10–30 cm or even more. In some genotypes, only the tip of the young shoot, not the complete shoot, died. In this case, the base of the shoot usually showed necrosis on the stem. Sporadically, the shoot tip wilt, some leaves close to the shoot tip died but the tip restarted to grow. Most of the plants showing this kind of reaction had necrotic leaves.

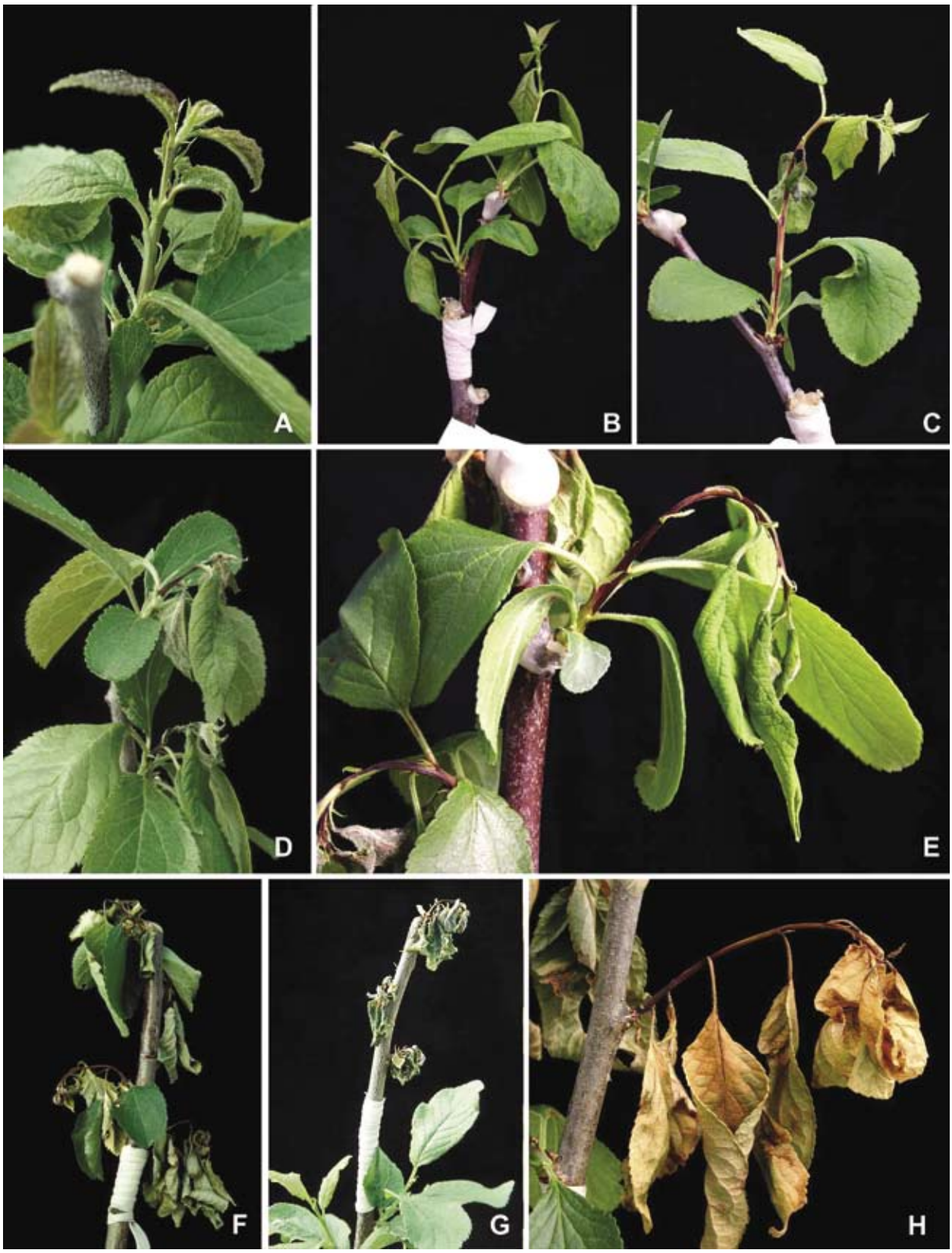

Fig. 1. Development of shoot tip necrosis (which results in the death of the young shoot tips) in hypersensitive genotypes after PPV inoculation using the double grafting method. A – Young leaves get olive green and reddish and commence wilting (rating value 1, clone Hoh 7460). B – Shoot tips are wilting (rating value 2, clone Hoh 7460). C – Shoot tip curves like an arc (rating value 3, clone Hoh 5944). Often, stem necrosis appears. D, E, F – Starting from the shoot tip, the scion shoot is dying back (rating value 4, clone Hoh 6372). G, H – The shoots have completely died off (rating value 5, clone Hoh 7342)

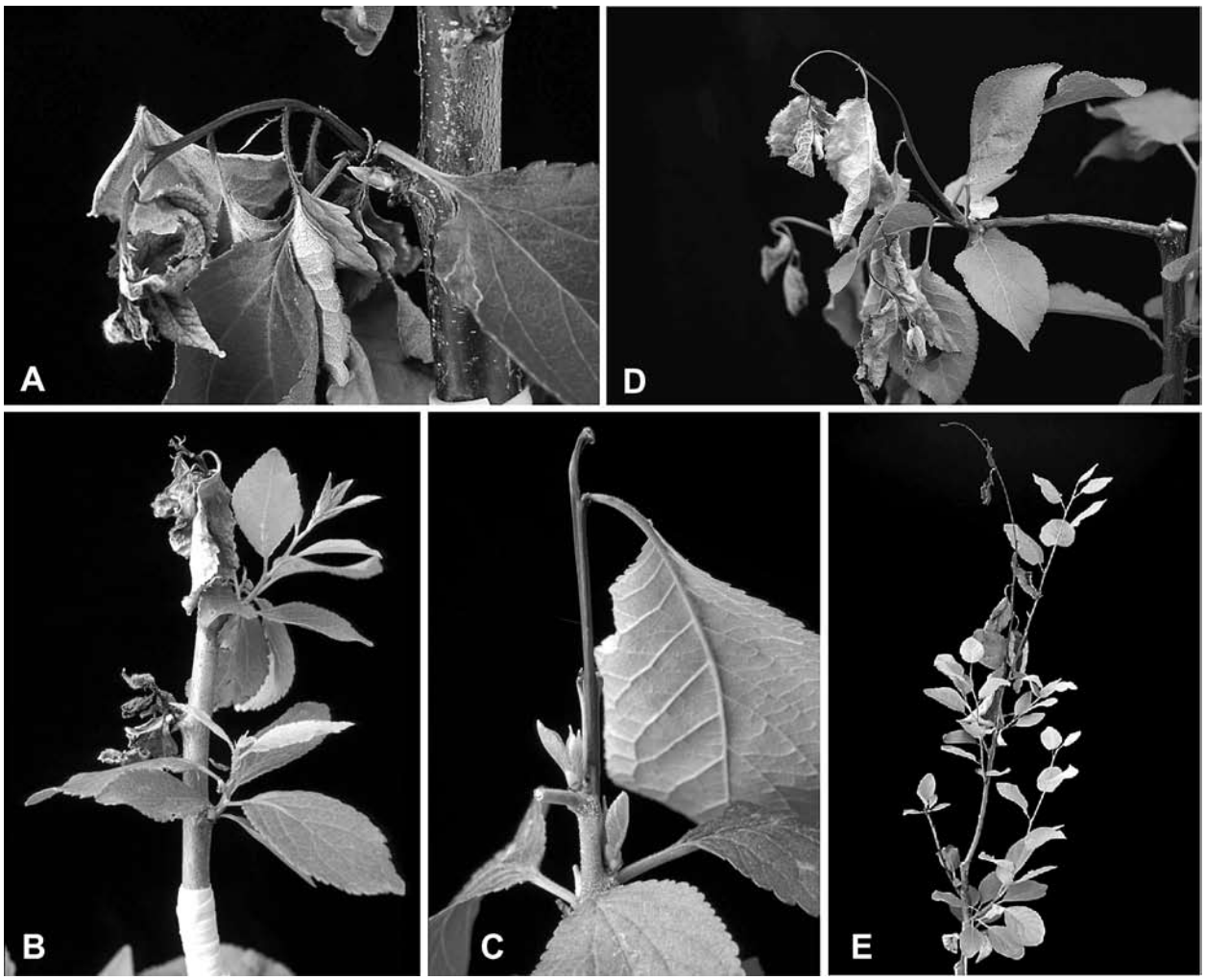

Fig. 2. Specific behaviour of some plum genotypes after artificial PPV inoculation using the double grafting method: A – After dying-off of all new scion shoots, a new bud is formed at the base of the dead shoot (clone Hoh 6372). B – The shoots growing out of the buds are free of PPV symptoms, no virus can be detected in the leaves (clone Hoh 6372). C – The same behaviour can be observed if the shoot tip is not dying off completely and new buds start to grow below the necrotic tissue of the scion shoot (clone Hoh 6029). D, E – After several months of growth (D) or after a dormancy period (E), initially the tips of some single shoots get necrotic. Later on, the whole scion graft is dying off (clone Hoh 5971)

Between the individual genotypes, there were significant differences in the time period after bud-break when the shoot tip necrosis appeared and in the intensity of this kind of necrosis. Fig. 1 gives an overview on the chronology of symptom development. Modifications of the typical symptomatology are shown in Fig. 2.

Necrosis on the stem of the shoots

In some genotypes, necrosis on the outer cell layers of the young shoots appeared. At first, it was visible as fine, brownish-black lines perpendicular to the shoot cross section area. These lines stretched out. In some cases, they reached from the base to the top of the shoot. Due to the disturbed growth, the shoot often curved into the direction where the stem necrosis appeared. This kind of distortion must not be confused with the one which appears when the shoot tip is dying off, primarily caused by the shoot tip wilting. Leaves whose petioles originated in a necrotic zone on the stem died off. Sometimes, the necrosis remained as thin as fibres, sometimes it finally covered the whole shoot, which led to the dying back of the upper part of the shoot. Sometimes, gum production was observed at the borders of the necrotic tissue.

In some single cases, the necrosis was visible only after the hardening of the shoot. In the periderm, rifts appeared which partially reached deep into the xylem tissue. In case of mild symptoms the periderm was scaled off in thin layers.

The development of necrosis usually started in the cambium and adjacent cell layers as could be

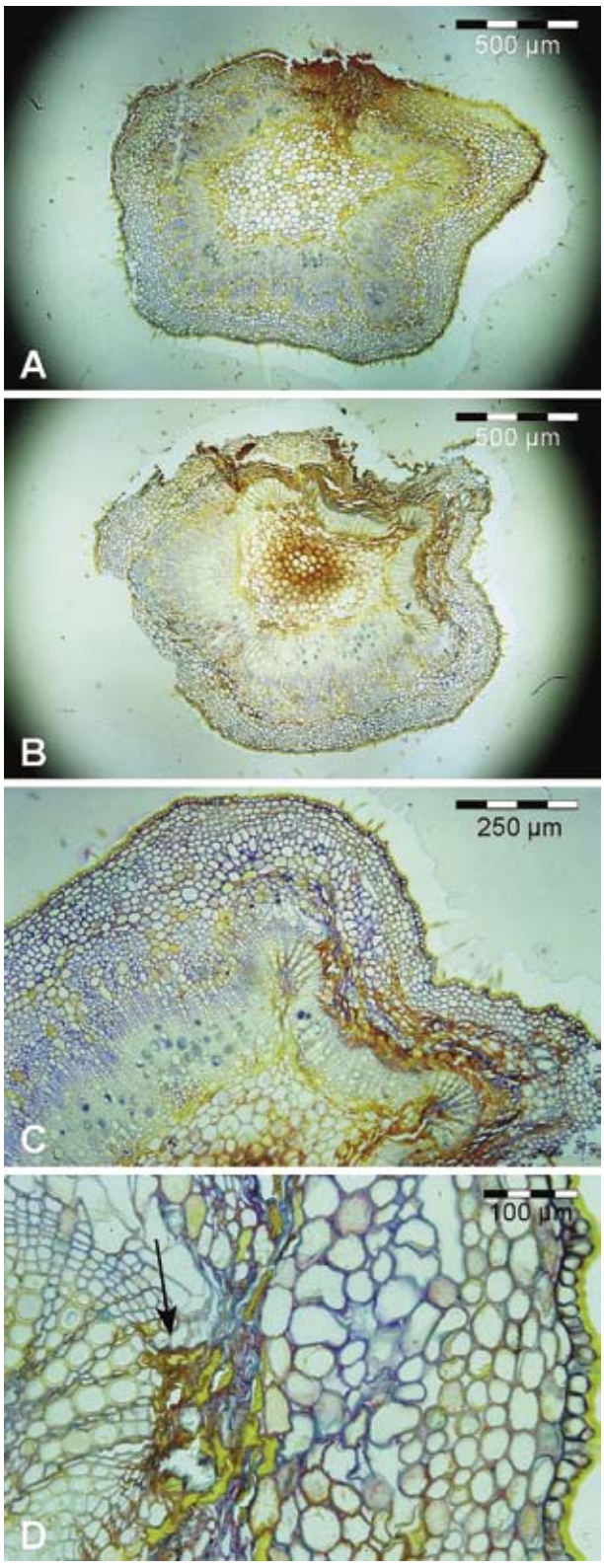

Fig. 3. Cross section through the stem of a young shoot with stem necrosis (Hoh 7355). A – Initial stage with first necrotic cells (arrow). B – Five days later: the region with the necrotic tissue has enlarged. On one half of the shoot, the tissue is collapsing. C – Transition tissue between healthy and necrotic tissue. D – Necrosis development starts at the cambial cells (arrow)

observed on histological sections. Cell death furthermore occurred in other cells in the cambial ring. Subsequently, phloem and epidermal cells died off. Even xylem vessels collapsed. In some genotypes, the necrosis finally covered the whole shoot. Fig. 3 shows the development of stem necrosis in young shoots.

Necrosis on the leaves

Most of the genotypes showed necrosis on the leaf blade. Often, it developed very rapidly so that no preceded chlorotic phase could be observed. The necrosis always developed near the veins, nearly in no case it originated in intercostal fields. Partially, the necrotic areas covered only a small part of the leaf blade. In this case, the necrosis was either point-shaped or lamellar, only very rarely both types could be observed on the same genotype (Fig. 4). Sometimes, the necrotization was preceded by a red colourization of the respective leaf areas. In most of the genotypes developing necrosis on the leaves were visible as irregular blots on the leaf blade which itself curled due to the death of whole tissue regions. In some cases, the necrotic spots enlarged so that most of the leaf blade got necrotic. Those leaves dropped down early.

PPV symptoms on the leaves

Some genotypes showed typical PPV symptoms on the leaves. In few cases, the typical light green rings and spots got heavily chlorotic and covered more than two thirds of the lamina (Fig. 5).

Modifications of the vegetative growth

Concerning the vegetative growth rate, two types of genotypes could be distinguished (Fig. 6):

(1) The growth rate and habit was not influenced. Especially the genotypes showing typical PPV symptoms on the leaves (rating values 1–5) and/or mild necrosis on the leaves and on the stem (rating values 1 and 2) were classified to this category.

(2) The vegetative growth rate was reduced, the internodes were shortened remarkably. Mainly genotypes showing medium or strong necrosis on the leaves and on the stem (rating value 3–5) showed this kind of reaction to artificial PPV inoculation.

These observations are based on comparisons of the entity of 1,329 PPV infected genotypes in three replications (3,957 plants) and not on comparisons with PPV free control plants.

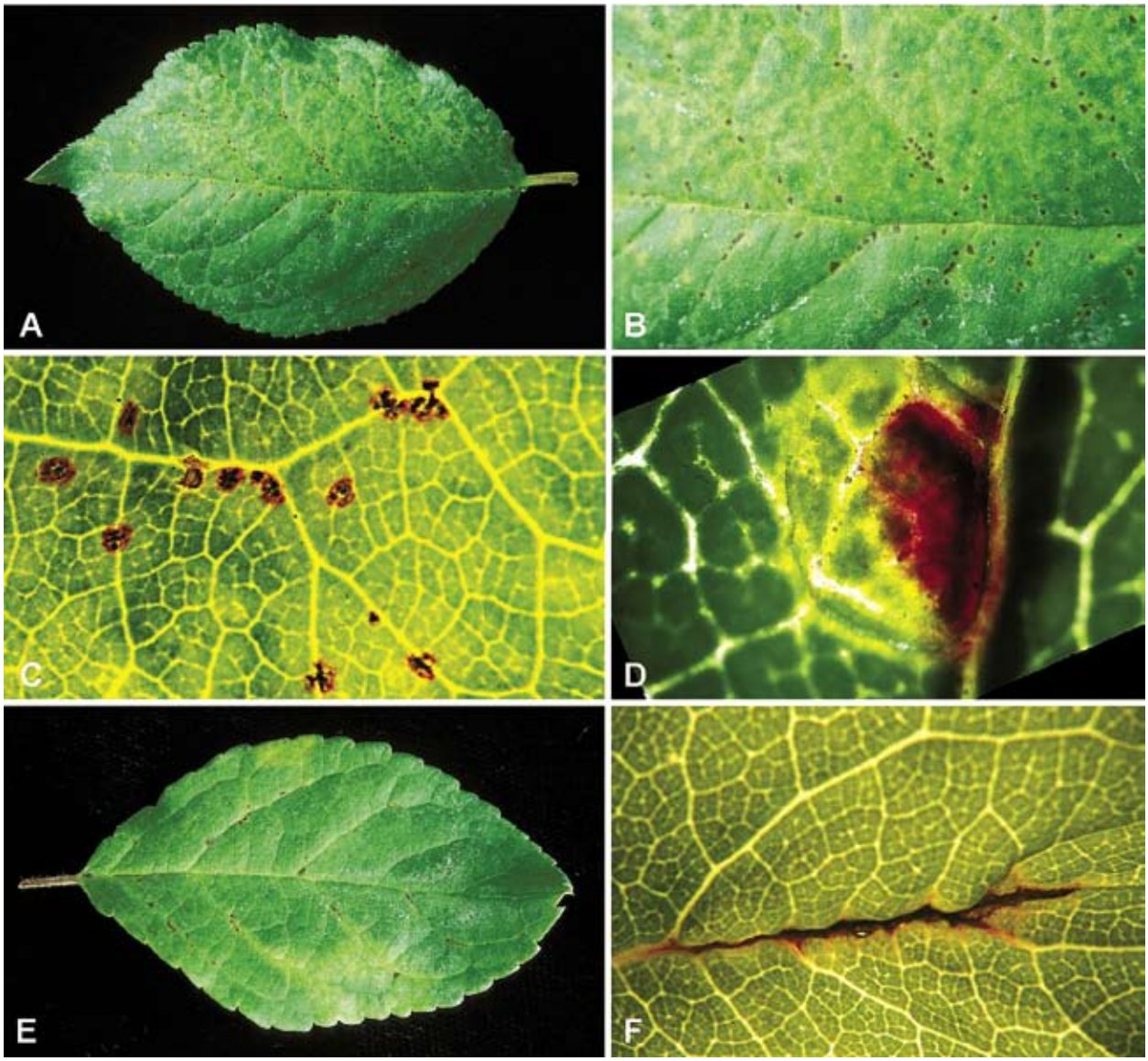

Fig. 4. Examples of weak necrosis on the leaf blade (rating value 1). The necrosis always develops near the veins. There is a sharp border to the healthy tissue. A, B, C, D – Point-shaped necrosis (Hoh 5306). E, F – Lamellar necrosis (Hoh 5310)

The genotypes grafted onto the trees in the field showed the same reaction to PPV as the genotypes tested in the greenhouse. The plants of all three replications developed similar symptoms. Data are given in the following section as the hypersensitivity index, which is derived from the observations, was used to compare the two test methods.

DISCUSSION

Hypersensitivity as a quantitative trait

The variability of the trait "hypersensitivity against PPV" is very high between single genotypes of *P. domestica*. In each of the progenies investigated seedlings showing typical PPV symptoms without any sign of hypersensitivity as well as seedlings with clear signs of strong hypersensitive response to PPV were found. Between these extremes, there was a group of genotypes showing necrosis on the leaves and/or on the stem, but without shoot tip necrosis. In a seedling population descended from the K4-hybrid, a genotype which shows hypersensitivity when infected with some PPV isolates, Kegler et al. (1991) observed different kinds of reactions to PPV infection as well. The authors only made two groups: genotypes with typical PPV symptoms and genotypes with necrotic reactions. The present results shows that in descendants of the hypersensitive clones Jojo and Ortenauer × Stanley 34, there is a larger variability in the seedlings concerning the

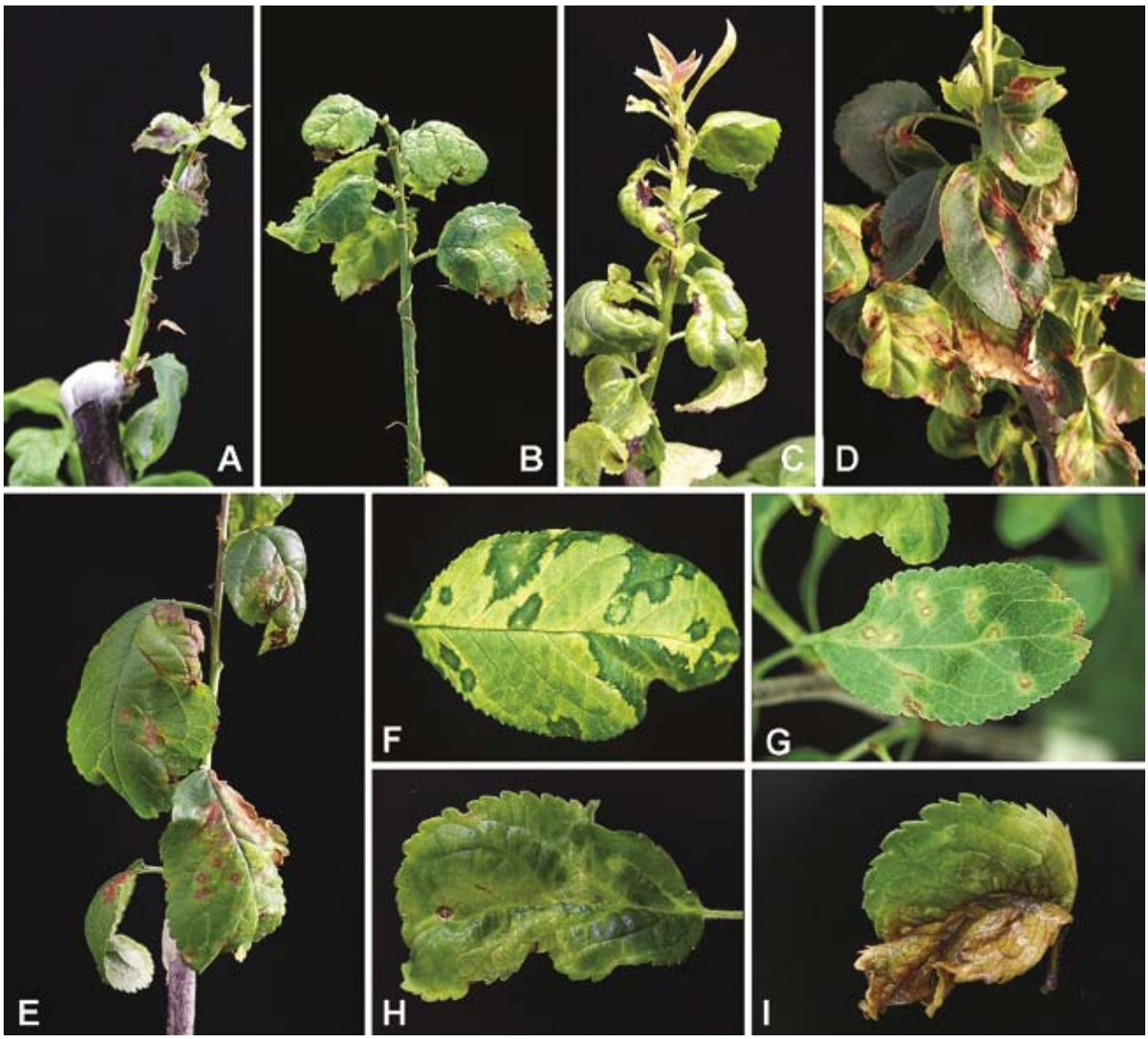

Fig. 5. Characteristic values of necrosis on the leaf blade. A, B – Strong leaf necrosis leads to the abscission of the respective leaves (Hoh 6742 and Hoh 7412). C – Rating value 3 (Hoh 7199). D – Rating value 5 (Hoh 6744). E – The shape of the necroses on one leaf can vary (Hoh 6789). F – Strong chlorosis on the lamina with no necrosis (Hoh 5355). G – Necrosis surrounding the typically chlorotic spots on the lamina (Hoh 7399, rating value 2). H – Some single necrosis on the lamina of Hoh 7558 (rating value 1). I – Large areas of the leaf blade get necrotic (Hoh 7409, rating value 5)

hypersensitivity trait which justifies the classification of the seedlings into four groups (see Table 3):

– Genotypes without *any sign of hypersensitivity* against PPV (hypersensitivity class 0)
  Usually, these genotypes show typical PPV symptoms on the leaves. In some single genotypes where no symptoms were visible, PPV could be detected either by ELISA or RT-PCR method. Hence, none of the investigated 1,329 hybrids could be proved to be immune to PPV. This finding goes along with the statements given by Hartmann and Petruschke (2000) and Grüntzig et al. (2001).
– Genotypes showing *weak hypersensitivity* against PPV (hypersensitivity class 1)
  Genotypes showing necrosis on the leaves and/or weak necrosis on the stem after strong PPV inoculation are called weakly hypersensitive. Most of them show PPV symptoms on the leaves as well. There is no death of the young shoot tips. Often, the leaves are curled and the vegetative growth is reduced (shortened internodes).
– Genotypes showing *moderate hypersensitivity* against PPV (hypersensitivity class 2)
  Those genotypes always show the death of the young shoot tips and very often, additionally, necrosis on the leaves. Typical PPV symptoms on the leaves are missing. The death of the shoot tip not always leads to the death of the whole young

Table 5. Comparison of the test systems – 'double grafting in the greenhouse' (GH) and 'grafting onto PPV infected trees in the field' by means of HI and HC based on the rating data (see Table 2)

Parentage	Clone Hoh	HI			HC		
		GH	field	difference[1]	GH	field	difference[1]
Hoh 4465 × Jojo	7909	0.00	0.00	0.00	0	0	0
	7918	0.93	0.87	0.07	3	3	0
	7935	1.00	0.96	0.04	3	3	0
	7938	1.00	0.93	0.07	3	3	0
	7945	1.00	0.93	0.07	3	3	0
	7957	1.00	0.93	0.07	3	3	0
	7960	1.00	0.89	0.11	3	3	0
	7973	0.94	0.94	0.00	3	3	0
	7975	0.82	0.94	0.12	3	3	0
Hoh 4515 × Jojo	8003	0.00	0.00	0.00	0	0	0
	8013	0.59	0.42	0.16	2	2	0
	8022	0.00	0.00	0.00	0	0	0
Elena × (Ort × Stan 34)	4543	0.87	0.90	0.03	3	3	0
	4601	0.77	0.74	0.02	3	3	0
	4608	0.90	0.78	0.12	3	3	0
	4632	0.90	0.52	0.38	3	2	1
	4648	0.93	0.93	0.00	3	3	0
Fellenberg × Jojo	6601	0.88	0.84	0.05	3	3	0
	6603	0.93	0.89	0.05	3	3	0
	6621	1.00	0.81	0.19	3	3	0
	6625	1.00	0.93	0.07	3	3	0
	6627	0.82	0.81	0.01	3	3	0
Hanita × Jojo	5910	0.74	0.79	0.04	3	3	0
	5911	0.72	0.90	0.18	3	3	0
	5912	0.80	0.90	0.10	3	3	0
	5925	0.83	0.79	0.04	3	3	0
	5928	1.00	0.88	0.12	3	3	0
	5933	0.86	0.83	0.03	3	3	0
	5942	0.80	0.88	0.08	3	3	0
	5944	0.95	1.00	0.05	3	3	0
	5953	0.90	0.07	0.83	3	0	3
Jojo × Hoh 4465	7736	1.00	0.87	0.13	3	3	0
	7761	0.60	0.00	0.60	2	0	2
	7762	0.80	0.87	0.07	3	3	0
Jojo × Fellenberg	7493	0.88	0.75	0.13	3	3	0
	7489	0.48	0.83	0.35	2	3	1
Jojo × Felsina	6404	0.03	0.05	0.02	0	0	0
	7350	0.15	0.13	0.02	1	1	0
Jojo × Hanita	6021	0.76	0.88	0.12	3	3	0
	6372	0.96	1.00	0.04	3	3	0
Jojo × Katinka	6726	1.00	0.77	0.23	3	3	0
	6759	0.06	0.10	0.04	0	1	1
Jojo × Clone 108	6868	0.08	0.10	0.02	0	1	1
	6869	0.03	0.00	0.03	0	0	0

Table 5 to be continued

Parentage	Clone Hoh	HI			HC		
		GH	field	difference[1]	GH	field	difference[1]
Jojo × Clone 128	6968	0.06	0.11	0.05	0	1	1
Jojo × Presenta	5276	0.85	0.89	0.04	3	3	0
	5278	0.00	0.00	0.00	0	0	0
	5350	0.46	0.85	0.39	2	3	1
	5357	0.79	0.80	0.01	3	3	0
Jojo × Zwintschers Frühe	6415	0.97	1.00	0.03	3	3	0
	6423	1.00	0.94	0.06	3	3	0
	6484	1.00	0.68	0.32	3	2	1
	6494	0.82	0.90	0.08	3	3	0
	6495	0.85	0.91	0.06	3	3	0
	6501	0.87	0.95	0.08	3	3	0
(Ort × Stan 34) × Hanita	4444	1.00	0.94	0.06	3	3	0
	4455	0.03	0.90	0.87	0	3	3
	4470	0.79	0.89	0.10	3	3	0
	4478	0.90	0.86	0.04	3	3	0
	4484	0.80	0.82	0.02	3	3	0
	4495	0.88	0.46	0.42	3	2	1
	4500	1.00	0.82	0.18	3	3	0
	4505	0.95	0.89	0.06	3	3	0
	4580	1.00	0.92	0.08	3	3	0
(Ort × Stan 34) × Jojo	6557	1.00	0.83	0.17	3	3	0
	6567	1.00	0.89	0.11	3	3	0
	6570	1.00	0.46	0.54	3	2	1
	6591	0.59	0.52	0.07	2	2	0
Mean				0.13			

[1]difference between the HI or HC, respectively, obtained from observations in the greenhouse and in the field

Fig. 6. Modifications in the growth habit of plum genotypes after artificial inoculation with PPV-D via double grafting. A – Normal vegetative growth of clone Hoh 7101 after inoculation with PPV-D. B – Shortening of the internodes and development of necrosis on the leaves (Hoh 7084). C – Shortening of the internodes and development of necrosis on the abnormally narrow leaves (Hoh 6241). D – Extreme shortening of the internodes (Hoh 7347)

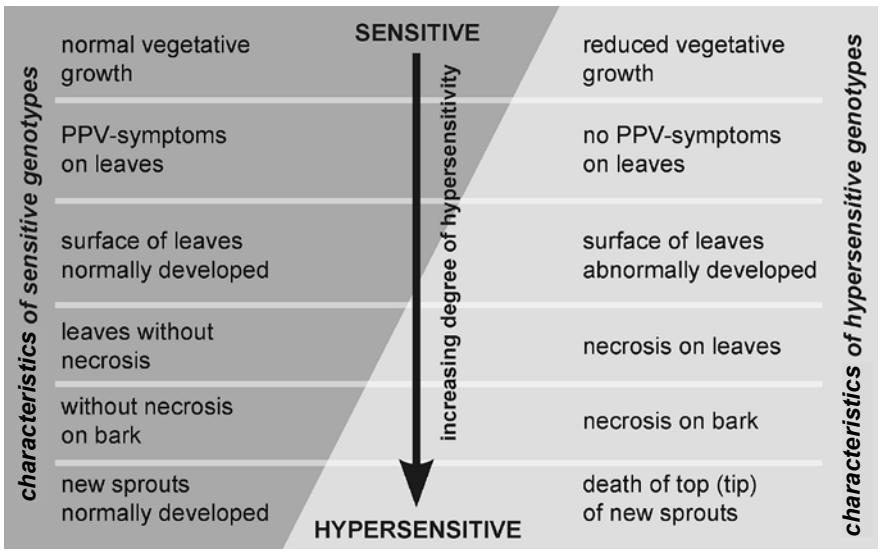

Fig. 7. The traits of hypersensitivity compared to the traits of sensitivity of *P. domestica* to PPV. The traits of hypersensitivity only get visible when the scion wood of hypersensitive genotypes is grafted onto PPV infected plants. The traits mentioned at the bottom of the figure contribute more to the hypersensitivity complex than those in the upper part

shoot, there may remain some healthy tissue on the basal part of the young shoot.

– Genotypes with *strong hypersensitivity* against PPV (hypersensitivity class 3)
  Genotypes classified into this group show a very fast death of the young shoots. They die off completely.

There is a smooth transition between the different classes of hypersensitivity (HC). It is evident that, from the phenotypical point of view, the hypersensitivity of European plum to PPV is a quantitative trait. This finding disagrees with the thesis postulated by Kegler et al. (2001). According to them, the hypersensitivity of *Prunus domestica* against PPV is a qualitative resistance and there is either a strong hypersensitive response to PPV infection or not. However, later on, the authors relativized their assumption by separating the mentioned K4-seedlings in several categories concerning their reaction to PPV inoculation. The presented data argue for an even more sophisticated differentiation concerning the hypersensitivity classes.

Fig. 7 gives an overview of the quantitative nature of hypersensitivity resistance to PPV and the characteristics which are used for defining the degree of hypersensitivity. As all individuals of a genotype react uniformly it can be stated that the differences are genetically determined.

Development of new shoots after the death of the young shoot tip

After the occurrence of the death of the young shoot tips, some genotypes developed new buds. The shoots which grew out of them either died off a few weeks later or did not show any symptom indicating either sensitivity or hypersensitivity over a period of several months. In the latter case, PPV could not be detected in the new shoots. This is the first report on such behaviour in European plum. It means that these genotypes must be able to prevent the movement of the virus from the PPV infected interstem into the respective tissue. However, this mechanism is not able to prevent the infection of the grafted hypersensitive genotype for a long time because sooner or later the new shoots develop necrosis and die off, sometimes only after a dormancy period. Nevertheless, it can be suggested that this kind of resistance level is able to prevent a natural infection with PPV in the orchard as in this case, there is no permanent and massive PPV infection pressure from an infected interstem as in the test system. Restrictively, it has to be mentioned that the development of new shoots after the death of the young shoot tip could not be observed in all plants of the same genotype. There seem to be some more unknown influencing factors.

Strength of reaction in comparison with Jojo

The phenotypically visible reaction of the hypersensitive and completely PPV resistant cultivar Jojo was described in detail by Hartmann (1997), Petruschke and Schröder (1999) and Hartmann and Petruschke (2002) and confirmed by Grüntzig et al. (2001) and Müller (2005). In the present study, Jojo showed the same reaction. However, there were genotypes that showed a much stronger and faster hypersensitive response to PPV inoculation, e.g. Hoh 6563 and Hoh 6587. Thus it can be stated that seedlings of Jojo and its sisterclone Ortenauer × Stanley 34 can show a higher degree of hypersensitivity than the hypersensitive parent cultivar itself (Table 4).

Setting up of an equation for calculating the hypersensitivity index

Based on the rating data of 1,329 genotypes of *P. domestica*, a tool was developed in order to compare the degree of hypersensitivity of different genotypes. In analogy to the disease index developed by Bivol et al. (1987), an index of hypersensitivity (hypersensitivity index, HI) has been set up. The rating data are ordinally scaled. The higher the rating data of each character the higher is the impact of this character on the level of hypersensitivity of the respective genotype.

Neumüller (2005) showed that only genotypes with a strong hypersensitive response are able to localize the virus in the field and to remain PPV free under natural infection conditions. Genotypes that show only leaf necrosis but no death of the young shoot tip in the test system are not able to prevent the spread of the virus within the plant.

From the rated parameters (Table 2) those essentially contributing to the degree of hypersensitivity were chosen: necrosis on the leaf blade, necrosis on the shoots and death of the young shoot tips. In the given order, these characters have more and more bearing on the hypersensitivity character (Fig. 7). Thus, the hypersensitivity index was calculated according to the following formula. The index has to be reliable, easy to be calculated based on as few phenotypically visible traits as possible and must not overestimate the degree of hypersensitivity of a respective genotype.

$$HI = (10 \cdot I(DST) + 2m_{NS}/n_{NS} + m_{NLB}/n_{NLB}) \times 13^{-1}$$

with

$$I(DST) = ((2m_{DST(d)} + m_{L(DST(d))}) \times (2n_{DST(d)} + n_L)^{-1} + (2m_{DST(h)} + m_{L(DST(h))}) \times (2n_{DST(h)} + n_L)^{-1}) \times 2^{-1}$$

where:

HI – hypersensitivity index (0; 1),
I(DST) – index of the death of the shoot tip (0; 1),
m – rated value (0; 1; 2; 3; 4; 5),
n – maximum of the rated value ever possible (in the present case: $n_x = 5$ in all cases),
indices – rated characters (see Table 2).

The equation for calculation of the HI is composed of three individual indices: the index of the death of young shoot tips (I(DST)), the index of necrosis on the shoots and the index of the necrosis of the leaf blade. Each of the individual indices can take a value in between 0 and 1 as the rating data are divided by the maximum value they can reach. Thus, the indices are relative values. The individual indices must be weighed according to their contribution to the total complex of hypersensitivity. Only in this way it is guaranteed that only genotypes with a high I(DST) are classified as strongly hypersensitive. The index of the death of young shoot tips (I(DST)) is weighed tenfold, the index of necrosis on the shoots is weighed twice and the index of the necrosis of the leaf blade is weighed once. This means that a genotype showing no symptoms of wilting or death of the young shoot tip can, per maximum, reach an HI of $(10.0 + 2.1 + 1) \times 13^{-1} = 0.23$, and a genotype showing, additionally, no symptoms of bark necrosis, an HI of 0.08 per maximum. The weighting factors were chosen in order to ensure the classification of the genotypes to the hypersensitivity classes mentioned above.

The I(DST) is calculated out of the rating data of four traits which all confer to the death of young shoot tips: the rating data of the healthiest and the most diseased shoot and the respective lengths. The rating value of the death of the young shoot tip is weighed twofold as the development of this symptom is more important for the description of the hypersensitivity index than the length of the shoot (Fig. 7).

For calculating the HI, the rating data of the third rating (12 weeks after potting) were used. Sometimes, the necrosis of the stem and the leaves could no longer be rated at that date because of the death of the respective shoots. In this case, the rating data of the earlier rating date for these characters were used. The HI of a genotype is the arithmetic mean of the HI of the three replications.

The HI of a respective genotype can take a value between 0 and 1. A value of 0 indicates that the plant does not show any symptoms of hypersensitivity. A HI of 1 is only possible in the case of all the traits contributing to hypersensitivity reaching their maximum values. Table 4 gives the HI and HC of some genotypes investigated.

The hypersensitivity index depends more on the genotype than on the environmental conditions of the plants. This has been shown comparing two test systems (testing under greenhouse and field conditions). In Table 5, the HI and the respective HC classification of 68 genotypes tested in both systems are shown. 56 genotypes (82.4%) were classified to the same HC, nine (13.2%) to the proximate HC. As the HC is based on the HI, such a variation has been expected. Three out of those nine genotypes had a difference of less than 0.05 between the HI based on the two test systems. Only in three cases (4.4%), the difference between the variants of a genotype has been so high that it led to the classification into the classes HC 0 or HC 1 in the one and into the classes HC 2 or HC 3 in the other test system, respectively. The mean deviation of

the difference in the HI was 0.13. There is no tendency of one of the two test systems to classify genotypes as more hypersensitive. Thus, both test systems are equally suitable for the determination of the degree of hypersensitivity of a respective genotype.

The use of the hypersensitivity index and the hypersensitivity class mirrors the quantitative character of the hypersensitivity of European plum against PPV. It harmonizes the results of tests for hypersensitivity. It enables the direct comparison of the degree of hypersensitivity of different genotypes. The formation of four hypersensitivity classes helps to decide whether a genotype is of interest for its PPV resistance in the field. According to the present knowledge, genotypes assigned to HC 3 remain free from PPV in the orchard under natural inoculation conditions. They can effectively localize the virus at the infection site. More research is needed to find out whether this is true for all genotypes assigned to HC 2 as well. Genotypes of HC 1 are not able to stop the spread of PPV within the plants. They are not recommended to be used as commercial varieties. The use of the hypersensitivity index is strongly recommended in breeding programs that aim at releasing new varieties hypersensitive to PPV. At least two varieties, Jojo (hypersensitive) and Common prune (sensitive to PPV), should be included in each hypersensitivity test. Moreover, in the forefront of releasing new varieties, long term field trials under natural inoculation conditions and the inoculation of the respective varietal candidate with a broad spectrum of virus isolates of different PPV strains are necessary.

List of symbols

PPV – *Plum pox virus*
NLB – necrosis on the leave blade
NS – necrosis on the stem of a young shoot (bark)
DST(d) – death of shoot tip on the most diseased shoot
DST(h) – death of shoot tip on the healthiest shoot
L(DST(d)) – length of the shoot with the most diseased shoot tip
L(DST(h)) – length of the shoot with the healthiest shoot tip
I(DST) – index of the death of the young shoot tips
HI – hypersensitivity index
HC – hypersensitivity class

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Fenotypicky-kvantitativní povaha hypersenzitivity vůči viru šarky u evropské slivoně (Prunus domestica L.) a její popis pomocí indexu hypersenzitivity

ABSTRAKT: U více než 1 300 semenáčků evropské slivoně, pocházejících z křížení, kde alespoň jeden z rodičů vykazuje hypersenzitivní rezistenci vůči viru šarky švestky (PPV), byla zkoumána jejich reakce na umělé naočkování tohoto viru metodou dvojitého roubování při použití nakaženého mezikmenu. Ukázalo se, že hypersenzitivní rezistence vůči tomuto viru je fenotypicky-kvantitativním znakem. Při testování se objevily různé symptomy od slabé nekrózy na čepeli listu až po odumření celých mladých výhonků. Byl navržen pojem „index hypersenzitivity", který určuje stupeň hypersenzitivní rezistence u jednotlivých genotypů. Jeho použití se velmi doporučuje jako nástroj selekce při šlechtění na rezistenci založené na hypersenzitivitě.

Klíčová slova: šarka; hypersenzitivita; rezistence; hypersenzitivní reakce; index hypersenzitivity; třída hypersenzitivity; rezistentní šlechtění; peckoviny

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