Europ.J.Hort.Sci., **68** (1), 45–49, 2003, ISSN 1611–4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

Response of Selected *Prunus* Rootstock Clones to Infection by *Prune dwarf virus* and *Prunus necrotic ringspot virus*

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Abstract

Due to the economic importance of sweet cherry production and virus tolerance, a range of rootstock clones (hybrids of *Prunus* spp.) has for some time been under study for response to infection by the pollen-borne *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV).

The plants were artificially inoculated by bark grafting using well-prepared inocula. Throughout the phases of preparation and investigation all the plants were grown in containers filled with sterilized soil-substrate and were kept under conditions excluding accidental infection.

Virus replication in the leaf tissue was checked for by DAS-ELISA. Data of growth parameters (leaf and shoot development) and virus symptom expression (description and evaluation of number of leaves showing symptoms compared to overall number of leaves) was recorded weekly.

Two types of virus response were observed and will be discussed for their epidemiological importance: a) tolerance as a tool to guarantee economically efficient cultivation e.g. of cherries under conditions where accidental infection has to be taken into account; b) hypersensitivity as a prerequisite to stop infection and save valuable plants (e.g. seed and scion source trees) under conditions where accidental infection is relatively unlikely but could have costly consequence in case of occurrence.

Zusammenfassung

Reaktionsmuster ausgewählter Genotypen von Kirschunterlagen auf eine Infektion mit *Prune dwarf virus* und *Prunus necrotic ringspot virus*. Auf Grund der wirtschaftlichen Bedeutung des Süßkirschenanbaus und der Toleranz gegenüber Kirschenringfleckenviren wurde eine Reihe ausgewählter Genotypen von Unterlagen (Hybriden aus Kreuzungen zwischen *Prunus*-Arten) Untersuchungen auf ihre Reaktion gegenüber einer Infektion mit den pollenübertragbaren Kirschenringfleckenviren (*Prune dwarf virus*, PDV und *Prunus necrotic ringspot virus*, PNRSV) unterzogen. Dazu wurden die Versuchspflanzen durch Rindenschildchenpfropfung mit einheitlichen Inokula (je 5 nur PDV, nur PNRSV bzw. beides) infiziert. Während der gesamten Vorbereitungs- und Beobachtungszeit wurden die Pflanzen in mit sterilisiertem Erdsubstrat gefüllten Containern unter infektionsfreien Bedingungen gehalten.

Die Virusreplikation im Blatt wurde durch wiederholten DAS-ELISA überprüft. Daten zu Wachstumsparametern (Blatt- und Triebentwicklung) und zur Ausprägung von Virussymptomen (Symptombeschreibung, Verhältnis symptomtragender Blätter zu Gesamtzahl an Blättern) wurden wöchentlich erhoben.

Bezüglich der Virusempfindlichkeit wurden zwei Reaktionsmuster beobachtet, die im Hinblick auf ihre epidemiologische Bedeutung diskutiert werden: a) Virustoleranz als ein Leistungsmerkmal von Unterlagen, das einen wirtschaftlichen Kirschenanbau auch in Gebieten, in denen mit einer zufälligen Infektion mit Kirschenringfleckenviren gerechnet werden muß, garantiert; b) Hypersensitivität als eine Möglichkeit in Vermehrungsbeständen (z.B. Samenspenderanlagen und Muttergärten), die durch aufwändige phytosanitäre Maßnahmen virusfrei gehalten werden, eine zufällige Infektion zu stoppen und auf diese Weise Pflanzenmaterial, dessen Wert in seiner Virusfreiheit liegt, zu erhalten.

Key words. Prunus rootstock clones – PDV – PNRSV – tolerance – hypersensitivity

Introduction

Worldwide there are numerous *Prunus* spp. rootstock clones available which have been carefully selected for economically valuable pomological traits. High quality standards of fruit tree planting material is guaranteed by national certification programmes. However in the orchard, mature flowering trees are prone to infection by the pollen-borne viruses *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV). Mild strains are pervasive (HOWELL and LANG 2001) and in most of the commercially used sweet cherry cultivars the infection remains latent; impact on yield may be variable (NEMETH 1986; DESVIGNES 1999) depending – among other factors – on scion-rootstock-interactions.

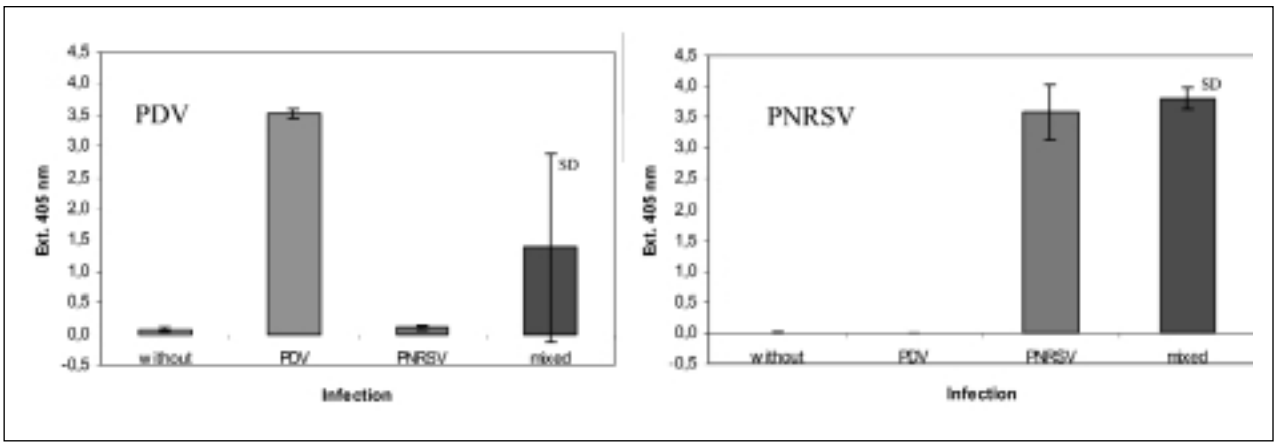


Fig. 1. Tolerant response type: results of DAS-ELISA for PDV, and PNRSV, resp., in leaves of 'Gisela 5' (70 d.a.i.) as shown in Fig. 2 (means of 5 plants per treatment; SD: standard deviation).

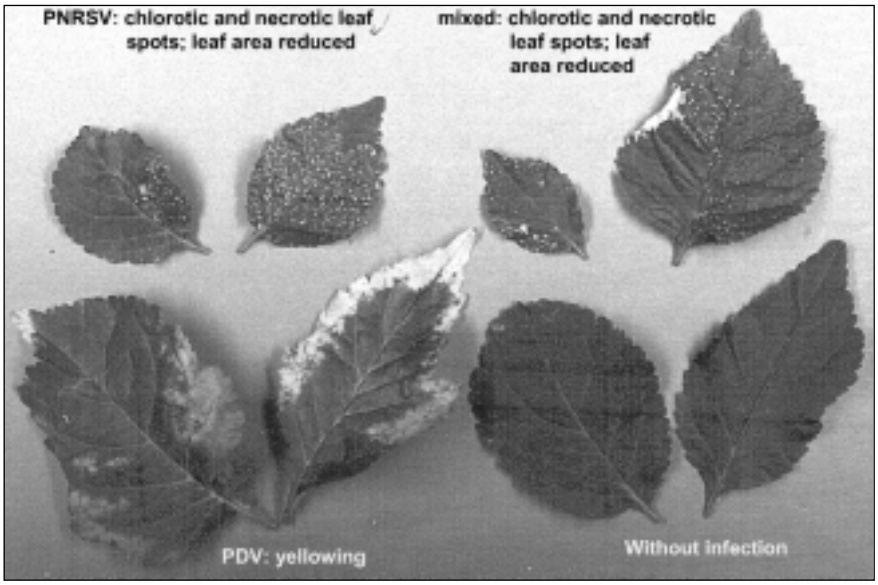


Fig. 2. Tolerant response type: symptoms on the leaves of 'Gisela 5' (70 d.a.i.) caused by 3 variants of virus infection (means of 5 plants per treatment; SD: standard deviation).

For several years now in the Pacific Northwest of the United States of America comprehensive projects have been in progress to study the effects of PDV and PNRSV infections on sweet cherry cultivars grafted on a large range of rootstocks (LANG, et al. 1997, 1998; HOWELL and LANG 2001; LANG and HOWELL 2001). For these studies the scion part of mature trees of different ages was inoculated and subsequent response of the grafted tree was observed. The results obtained were used for virus sensitivity classification of the rootstocks used for grafting. There is general agreement that only rootstock clones with „tolerant" response to the viruses mentioned above are of any economic interest. In this context „tolerance" means that there are no or only slight losses in growth, productivity as well as fruit quality, and at least in the first growing season no symptoms expressed by the scion cultivar.

To speed up classification of rootstock clones in our studies, similar to the method used by UPHOFF (1991), young rootstock liners were inoculated directly, and the response of the rootstock plant itself was recorded. For the purpose of rootstock classification the terms „tolerant" and „hypersensitive" are used according to UPHOFF'S (1991) definition. Further differentiation is made between tolerance based on performance and tolerance based on virus symptom expression as NIENHAUS (1985) suggested.

Materials and Methods

In preparation for the studies, virus isolates common to European growing regions were collected with support of various institutions dealing with tree fruit virology. To achieve homogenity and compatibility they were propagated on *Prunus avium* 'F 12/1'. Bark chips of 30 mm length without buds were used for bark-graft inoculation at the stage of bud burst in one-year-old rootstock plants potted in 21 containers filled with sterilized soil-substrate. For up to 70 days after infection (d.a.i.) the plants to be tested were kept in the greenhouse under conditions preventing accidental virus infection.

The first course of the studies covered the cherry rootstock clones 'Gisela 5' (148/2), 'Gisela 6' (148/1), 'PiKu 1', 'PiKu 3', 'PiKu 4', 'VSL-2', the plum/prune

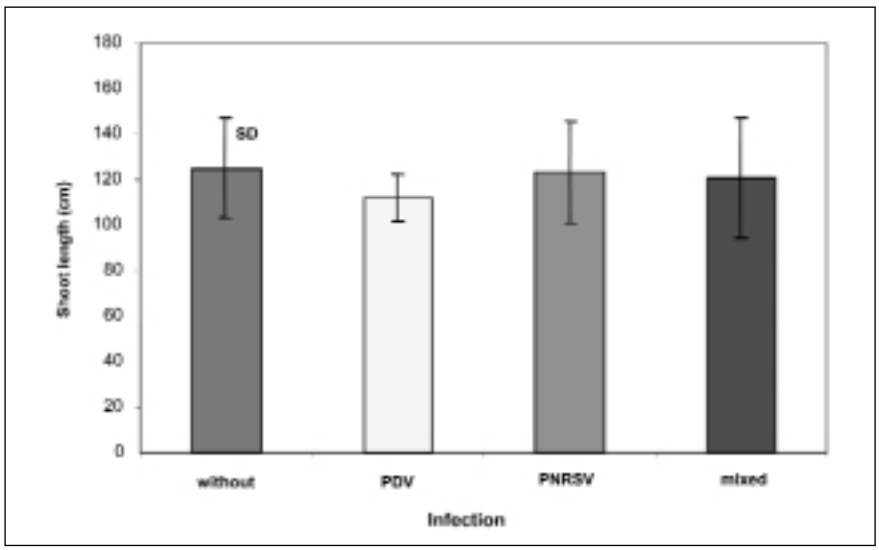


Fig. 3. Tolerant response type: shoot length of 'Gisela 5' (70 d.a.i) developed under 3 variants of virus infection (means of 5 plants per treatment; SD: standard deviation).

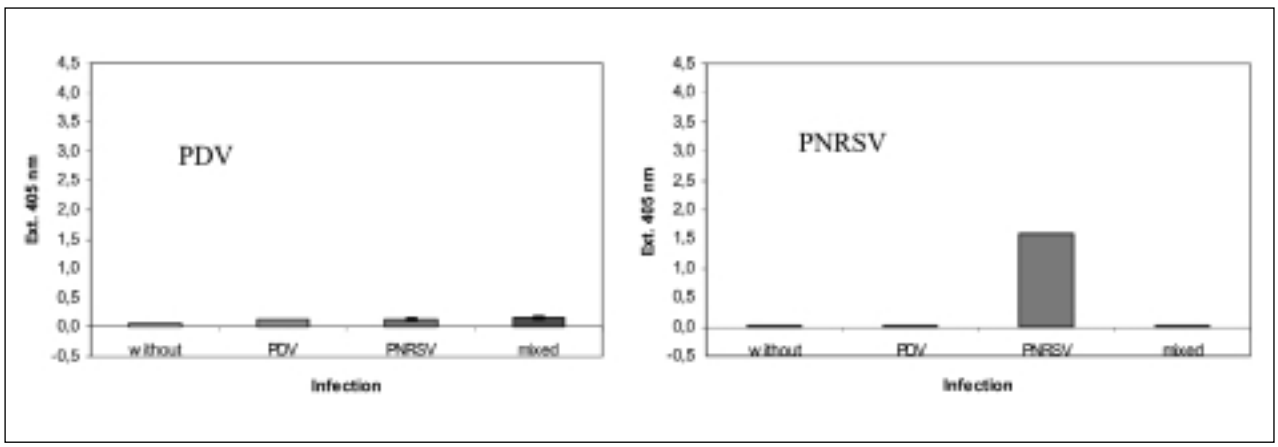


Fig. 4. Hypersensitive response type: results of DAS-ELISA for PDV, and PNRSV, resp., in leaves of 'VSL-2' (70 d.a.i.) without symptoms (means of 5 plants per treatment; SD: standard deviation).

clone 'VVA-1', as well as the well-known standards 'Colt' und 'F 12/1'.

Virus replication in the leaf tissue was checked for by repeated DAS-ELISA (70 and 230 d.a.i.). Quality (description, photography) and quantity (ratio of leaves with symptoms to overall number of leaves) of leaf symptoms was recorded weekly. To gain information on possible growth reductions, the number of leaves were counted and length of shoots was measured.

Results and Discussion

Rootstocks with tolerant response to pollen-borne viruses had high virus replication in the leaf tissue. This was true for both viruses (Fig. 1). They showed various kinds of leaf symptoms (Fig. 2), and between 10 and 40 per cent of the leaves expressed symptoms. No growth reductions were observed (Fig. 3).

Only one rootstock was characterized by hypersensitive response. This one displayed very low replication of only PNRSV in the leaf tissue (Fig. 4). Neither leaf symptoms nor growth reductions (Fig. 5) were observed. However eventually (between approx. 120 and 150 d.a.i.) all the plant parts apical to the point of virus inoculation were lost by lethal necrosis. The tissue basal to the bark graft remained vital and, as subsequently axial shoots developed, the individual plant survived the virus infection (Fig. 6).

Based upon these findings the virus response type of the *Prunus* spp. rootstock clones tested have been classified as follows: as already known, the standards 'Colt' und 'F 12/1' proved to be tolerant as did 'Gisela 5', 'PiKu 1', 'PiKu 3' and 'PiKu 4'. Due to reductions in shoot growth and leaf development, respectively, 'Gisela 6' and 'VVA-1' are referred to as „partially sensitive". Only 'VSL-2' was characterized as „hypersensitive".

For 'Gisela 6' this is in contrast to the results of HOWELL and LANG (2001) as well as LANG et al. (1997; 1998), LANG and HOWELL (2001), and UPHOFF (1991). Comparing test design and methods used, this is obviously due to the heavier virus pressure and the observation period being restricted to the shock phase of the disease in our studies. The category of 'Gisela 5' conforms with the grading by the authors mentioned above. For the other clones there is no information available in literature.

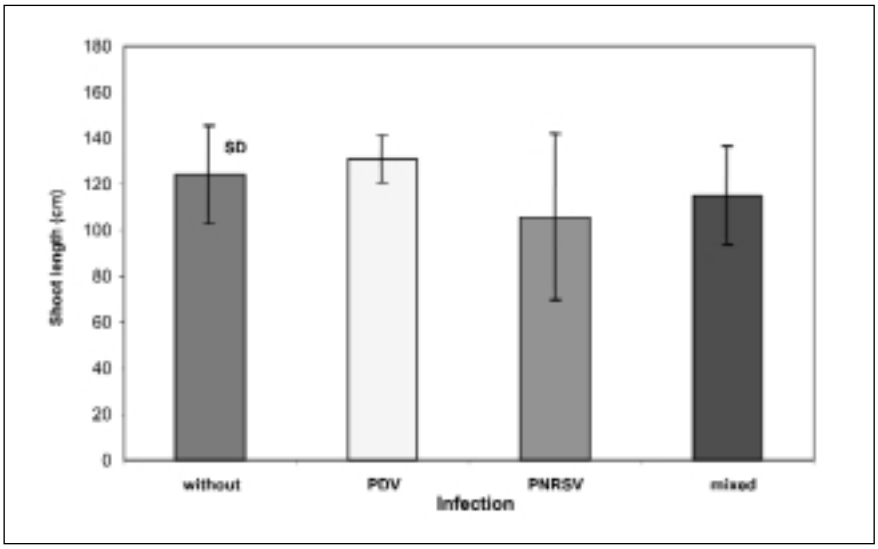


Fig. 5. Hypersensitive response type: shoot length of 'VSL-2' (70 d.a.i.) developed under 3 variants of virus infection (means of 5 plants per treatment; SD: standard deviation).



Fig. 6. Hypersensitive response: necrosis and decline of the shoot apical to the place of virus inoculation (arrow), sprouting of shoots at basal, non-infected parts of the shoot.

Conclusions

Since classification of *Prunus* spp. rootstock clones according to their virus response type is of great economic importance, early results are valuable. As could be shown, classification on the basis of results gained through short term studies during the shock phase of the disease, with rootstock plants infected directly, is highly selective due to high virus pressure. Therefore, the range of clones designated for further long term studies with grafted trees in the orchard can be reduced, scaling down research expenses.

Furthermore the epidemiological importance of the virus response type should be stressed. Since virus tolerant plants do not show significant losses in vigour, productivity and fruit quality when infected, an economically efficient cultivation is possible even under virus pressure. However, the infected plant will act as a virus source and virus spread is supported in the orchard and in the region beyond. In contrast, if infected hypersensitive responding genotypes suffer from decline effects, detrimental losses of plant parts or even individual plants have to be taken into account. Cultivation is therefore only possible under virus-free conditions which are realized by sophisticated phytosanitary precautions in propagation stocks. In the rather unlikely case of accidental infection under those conditions, hypersensitive responding plants could prevent further virus replication and spread. This could be of great economic advantage in saving valuable plantations (e.g. seed and scion source trees respectively), preventing a loss of entitlement to production of certified plant material and cutting down expenses for comprehensive virus testing.

Acknowledgements

The studies were sponsored by „Stiftung Gisela“. This financial support as well as material support by all the colleagues who provided virus inocula is gratefully acknowledged.

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Received October 02, 2002 / Accepted December 17, 2002

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