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Hot-water treatment of dormant grape cuttings: Its effects on *Agrobacterium tumefaciens* and on grafting and growth of vine[1])

by

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Heißwasserbehandlung von dormantem Rebschnittholz: Einfluß auf *Agrobacterium tumefaciens* sowie auf die Pfropfung und das Wachstum von Reben

Zusammenfassung: 40 cm lange Stücke des 1jährigen Holzes von vier Ertrags- und vier Unterlagsrebsorten wurden mit heißem Wasser (20 bzw. 30 min bei 50 °C) behandelt, um die Möglichkeit der Thermotherapie zur Bekämpfung von *Agrobacterium tumefaciens* Biovar 3 zu prüfen.

Nach der Antreibphase wurde Kallusmaterial von Stecklingen der Sorten Albana, Lambrusco Grasparossa, Ruländer und Fortana sowie ihrer Pfropfkombinationen mit den Unterlagen 420 A, 41 B, Kober 5 BB und 1103 Paulsen analysiert. Hierbei wurde nur eine niedrige Kontaminationsrate des verwendeten Rebenmaterials festgestellt. Ähnliche Ergebnisse zeigten in den USA auf die gleiche Weise behandelte Ertrags- und Unterlagensorten. Trotz gelegentlicher Kontamination erwies sich die Thermotherapie als wirksames Mittel zur Bekämpfung von *A. tumefaciens*.

Die Wachstumsparameter des behandelten Materials — bei Gewächshaus- und nach 8monatiger Freilandkultur — wurden ebenfalls ausgewertet. Die Wirkung der Heißwasserbehandlung auf Vitalität und Wachstum der Reben variierte in Abhängigkeit von der Edelreis-Unterlagenkombination. Die Behandlung beeinflußte die Vitalität im großen und ganzen nicht nachteilig; die Anzahl der vermarktungsfähigen Pfropfreben konnte jedoch verringert sein. In den meisten Fällen nahmen die Anzahl und Länge der Triebe sowie der Stammdurchmesser zu.

Die Wärmetherapie beeinflußte die Überlebensrate der Knospen im allgemeinen nicht, sie stimulierte jedoch in den meisten Fällen die Kallusbildung der Stecklinge.

Key words: crown gall, Agrobacterium tumefaciens, variety of vine, rootstock, cane, shoot, callus, latent infection, thermotherapy, growth, mortality.

Introduction

In recent years, there has been increasing interest amongst researchers and grape growers, in particular nurserymen, in grapevine crown gall caused by *Agrobacterium tumefaciens*. This is a result of the progressive diffusion of *Vitis* spp. in new viticultural areas and of the disease in European and extra-European states (FERREIRA and VAN ZYL 1986; OPHEL *et al.* 1988; BIEN *et al.* 1990), where it often had been considered occasional or even rare.

In grape the disease is generally associated with biovar 3 (AT3) of the pathogen (KERR and PANAGOPOULOS 1977), which can endophytically colonize the plant xylem,

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even in the absence of visible disease symptoms. The pathogen is systemically translocated in the plant sap and is unevenly distributed within the plant (BURR *et al.* 1983; TARBAH and GOODMAN 1986; STEFANI and BAZZI 1990).

The most important source of inoculum for long-distance spreading of AT3 may therefore be symptomless propagation material, which can harbour high levels of bacteria. Shoot-tip propagation of grapevines and the production and repeated indexing of herbaceous cuttings (BURR *et al.* 1987; GOODMAN *et al.* 1987) are effective but lengthy methods of obtaining AT3-free vines. An alternative and relatively easy method that has been proposed for eradicating the pathogen from propagation material is hot-water treatment. Dormant grape cuttings are submersed in a water bath at 50 °C for 30 min. Experimental evidence has shown that this method can be effective (BURR *et al.* 1989). It is now important to add large-scale trials on a greater number of varieties; to establish its potential for eradicating AT3; its effects on plant vitality and growth in the nursery, and the effect on grafting of vines. The objectives of this study were to test the effectiveness of hot-water treatment from a phytosanitary point of view and to assess its applicability as a routine nursery technique.

Materials and methods

Plant material

Four rootstocks were used: Kober 5BB (5BB) and 420A (*V. berlandieri* × *V. riparia*), 1103 Paulsen (1103P) (*V. berlandieri* × *V. rupestris*) and 41B (*V. vinifera* × *V. berlandieri*).

Cuttings were collected from dormant symptomless rootstocks in the Rauscedo Cooperative Nurseries (Northern Italy). Four *V. vinifera* cultivars were also used: Albana, Lambrusco Grasparossa, Ruländer and Fortana. Canes were taken from dormant vines with evident crown gall symptoms in the Modena, Bologna and Bolzano districts. The cuttings were taken at different heights on the vines and, where possible, close to galls. All the plant material was cut into 40 cm sections with an average of 4 nodes each.

A total of 420 cuttings were collected for each of the four rootstocks; after treatment, 360 of them were grafted (240 to assess growth after hot-water treatment and 120 for bacterial isolation from the callus at the graft union) and 60 were evaluated for the presence of AT3 in the basal callus. Hot-water treatment was also performed on 150 cuttings of each cultivar; 90 (corresponding to 360 buds) were grafted and 60 used to detect AT3 in the callus.

Additional experiments were carried out in 1989 and 1990 in the USA with cuttings obtained from Forster's Nursery in Fredonia, New York. Cultivars Niagara (*V. labrusca*), Ravat, Seyval blanc, Vidal blanc (interspecific hybrids) and rootstocks 5BB, 3309C and SO4 were used. Cuttings were obtained from vineyards known to be virus-free. They were approximately the same size as those used in Italy and were grouped in bundles of 100. 1000 cuttings of each cultivar or rootstock were heat-treated. Equal numbers of untreated cuttings were used as controls.

Hot-water treatment

For the experiments carried out in Italy, the cuttings were divided into three groups, the first group being the control. The other two groups were submersed in a tank of water maintained at 50 °C (4 bundles of 20 each) to avoid temperature variations. Submersion time was 20 min for the second group and 30 min for the third group. The cuttings were dried at room temperature treated with methyl thiophanate ($0.7\ g \cdot l^{-1}$) and placed in cold storage at 4 °C.

For the experiments done in the USA at least 1000 cuttings were treated at a time in a tank holding > 4000 l of water. In 1989, cuttings were treated on February 15th and in 1990 on March 6th. Water was heated in a boiler and the temperature was regulated by a thermostatically controlled pump. Temperature of the water bath and the internal temperature of cuttings were monitored with a thermocouple recorder. In 1990, two probes (copper and constantan wire) were placed at opposite ends of the water tank and three probes recorded the internal temperatures of cuttings at different locations in bundles and in the tank. Probes were inserted into the pith region of cuttings by drilling a 1.5 mm diameter hole, inserting the probe and sealing the wire with a thermoinsulating sealer. The time required for the internal tissues of cuttings to reach 50 °C was determined.

Grafting

The effect of heat-treatments on grafting was measured for the cuttings treated in Italy. Bench grafts were made 10 weeks after treatment with 1-bud scions. After grafting the upper part of the grafted cutting was dipped for a few seconds in paraffin (75 °C, red type, Stähler, Germany) containing a wound-healing hormone and the antifungal compound Chinosol. The grafting apparatus was disinfected with 70 % ethanol after each sample. The grafted and ungrafted vine material was placed in forcing boxes containing sterile fir sawdust moistened with water. The boxes were kept in forcing chambers at 34 °C for 48 h and subsequently at 30 °C for 2 weeks. Finally, after 2 d at room temperature, they were placed in the open air.

1 month later, the material was removed from the forcing boxes; the grafted material was waxed, as previously described, using no. 9174 ARCO paraffin.

For each graft combination, 20 vines were transplanted to a field nursery. A total of 20 cuttings for each cultivar, 20 for each rootstock and 10 vines for each graft combination were checked for AT3 in the callus at the graft union and at the base of the cutting of the ungrafted material.

Experimental field

A plot was selected in the S. Martino di Rauscedo locality, with homogeneous characteristics, where vines had not been grown for 10 years. The vines were planted out in a completely randomized design along a single row.

AT3 detection

In Italy, one group of treated material was indexed for AT3 immediately after the forcing period. Another was grown in the field and assayed in late fall. The method of Burr *et al.* (1989) was used for basal and graft callus indexing using a medium (RS) that is semi-selective for AT3 (Roy and Sasser 1983). Three colonies for each isolation resembling AT3 were purified on the same medium and subsequently transferred to King's medium B (King *et al.* 1954). Non-fluorescent colonies were then grown on PDA at 28 °C for 48 h, identified with a dot immunobinding assay, using the anti-AT3 Ab F21.ID3G7C8 monoclonal antibody of Bishop *et al.* (1989) and by performing pathogenicity tests on *Nicotiana glauca*.

Cuttings that were used in the experiments in the USA were indexed with the same methods.

Evaluation of growth parameters

The growth of the various graft combinations was assessed at harvest by checking graft-take, measuring the diameter of the rootstock immediately below the graft union,

calculating the number and length of the canes on the rootstocks and on the cultivars, and measuring the diameter of the graft union hyperplasias.

Analysis of variance was performed on the growth parameters. The Student t-test was chosen to compare treated and untreated plants for each cultivar and each scion/rootstock combination.

Cuttings treated or untreated in USA were rooted in perlite in the greenhouse for about 4 weeks. The number of viable buds were counted and the relative amount of callus production was rated on a 0 to 3 scale (0 = no callus and 3 = greatest amount).

Results

Italian experiments

The analyses carried out on the untreated (control) and treated materials led to the isolation and selection of more than 2000 bacterial colonies, including 385 that were non-fluorescent. Only 23 of the latter group were identified as AT3 using the dot immunobinding assay and the pathogenicity test.

These results indicate that the level of infection in the grape material used for the experiments was very low and, in most cases, below a detectable level, even in the cuttings taken from naturally infected vine cultivars with evident symptoms of crown gall on the aerial parts (Table 1). Analysis of the callus from control cuttings was negative for Lambrusco Grasparossa, while in the rootstock cuttings, taken from apparently healthy mother-vines, AT3 was found only in 1103P.

Table 1

Efficacy of heat-treatment for eradicating *Agrobaterium tumefaciens* biovar 3 from four grape cultivars and four rootstocks

Wirkung der Wärmebehandlung gegen *Agrobacterium tumefaciens* Biovar 3 bei vier Ertrags- und vier Unterlagenrebsorten

	Frequency of infected cuttings [1] (Treatment times at 50 °C)		
	0 min	20 min	30 min
Albana	2/20	0	0
Lambrusco G.	0	0	0
Ruländer	2/20	0	0
Fortana	2/20	0	1/17
420A	0	0	1/16
41B	0	0	0
5BB	0	0	0
1103P	2/18	0	0

[1]) Number of cuttings with AT3/number of cuttings assayed using callus method as described in the text.

No AT3 was found in the cuttings treated for 20 min; however, in the cuttings treated for 30 min the pathogen was only isolated twice, from a Fortana and from a 420A cutting.

Analysis of callus from 16 graft combinations, after the forcing period, did not lead to the isolation of AT3 in any of the treated material. In the untreated controls, 7 graft

Table 2

Efficacy of heat-treatment for eradicating *Agrobacterium tumefaciens* biovar 3 from 16 cultivar/rootstock combinations

Wirkung der Wärmebehandlung gegen *Agrobacterium tumefaciens* Biovar 3 bei 16 Kombinationen von Ertrags- und Unterlagenrebsorten

Combinations	Frequency of infected vines [1] (out of 10) (out of 20)		Discard frequency [2]	
	No heat	Heat	No heat	Heat
Albana				
420A	1	0	6	7
41B	0	0	1	7
5BB	0	0	4	11
1103P	2	0	2	3
Lambrusco G.				
420A	0	0	3	9
41B	1	0	2	4
5BB	1	0	2	2
1103P	0	0	6	4
Ruländer				
420A	0	0	4	6
41B	4	0	6	8
5BB	1	0	3	7
1103P	0	0	0	3
Fortana				
420A	1	0	10	8
41B	0	0	6	3
5BB	0	0	6	9
1103P	0	0	11	3

[1]) As determined by indexing methods described in the text.
[2]) Refers to grafts that were weak and not commercially acceptable and to dead vines.

combinations were infected by AT3, with frequencies varying from 1 to 4 grafted vines out of 10 (Table 2).

Table 2 also shows that hot-water treatment influenced grafting efficiency and vine mortality. In 11 graft combinations the discarded vines increased after treatment, sometimes quite markedly, as, for example, with Albana and 5BB. On the other hand, in Fortana heat-treatment reduced the incidence of discarded vines. Table 3 shows in more detail that hot-water treatment did not increase vine mortality except Albana on 41B, 5BB and 1103P and Lambrusco Grasparossa on 41B. Fortana graft combinations showed less mortality than treated vines. The heat-treatment, in general, had a negative effect on grafting efficiency, particularly on Albana and Ruländer and in the 5BB combinations. In these combinations, though there was good graft-take in 90—100 % of the controls, following heat-treatment for 30 min graft-take fell to 60 %, e.g. in the combination Albana/5BB. An improved graft-take was only observed in Lambrusco Grasparossa and Fortana on 41B and 1103P.

Analysis of variance of the data concerning the number and length of canes revealed a significant ($P < 0.01$) cultivar/heat-treatment interaction (Table 4). In Fortana the

Table 3

Influence of heat-treatment (30 min at 50 °C) on mortality in the field and on graft-take of 16 grape cultivar/rootstock combinations

Einfluß der Wärmebehandlung (30 min bei 50 °C) auf die Sterblichkeitsrate im Freiland und auf die Pfropfausbeute bei 16 Kombinationen von Ertrags- und Unterlagenrebsorten

Combinations	Frequency of dead vines [1]		Frequency of graft-take [2]	
	No heat	Heat	No heat	Heat
Albana				
420A	5/20	4/20	14/15	13/16
41B	1/20	4/20	19/19	13/16
5BB	4/20	5/20	6/16	9/15
1103P	1/20	2/20	18/19	17/18
Lambrusco G.				
420A	3/20	3/20	17/17	11/17
41B	0/20	4/20	18/20	16/16
5BB	1/20	0/20	18/19	18/20
1103P	3/20	2/20	14/17	16/18
Ruländer				
420A	4/20	4/20	16/16	14/16
41B	5/20	5/20	14/15	12/15
5BB	3/20	2/20	17/17	13/18
1103P	0/20	0/20	20/20	17/20
Fortana				
420A	10/20	1/20	10/10	12/19
41B	3/20	2/20	14/17	17/18
5BB	4/20	3/20	14/16	11/17
1103P	4/20	0/20	9/16	17/20

[1]) Each entry consists of 20 vines.
[2]) Number of commercially acceptable grafts/number of living vines.

Table 4

Influence of heat-treatment (30 min at 50 °C) on growth parameters of four grape cultivars

Einfluß der Wärmebehandlung (30 min bei 50 °C) auf die Wachstumparameter von vier Ertragsrebsorten

	Canes * (no.)		Cane length * (cm)		Graft union dia. (mm)		Trunk dia. (mm)	
	No heat	Heat	No heat	Heat	No heat	Heat	No heat	Heat
Albana	1.2	1.2	31.9	27.4	16.1	16.2	**8.6**	**9.2**
Lambrusco G.	1.4	1.6	**28.9**	**34.7**	16.3	16.3	8.8	9.3
Ruländer	1.4	1.3	33.1	33.6	15.1	15.2	8.3	8.6
Fortana	**1.3**	**1.7**	**27.4**	**38.1**	14.8	15.4	8.7	9.1

* Significant at $P < 0.01$ (F-test).
Significant ($P < 0.05$; t-test) mean values of heat-treated and untreated plants, within each cultivar, are given in bold type.

number of canes was significantly higher in the heat-treated plants than in the control; moreover, a marked and statistically significant increase of cane length was observed for Lambrusco Grasparossa and, again, for Fortana. As regards the graft union and trunk diameters, the cultivars showed a similar response to heat-treatment with a general tendency for an increase in both, particularly in Albana and Fortana.

Examining the variety/rootstock/treatment interactions, highly significant differences ($P < 0.01$) are observed as regards the graft union and trunk diameters of the grafted vines. Table 5 shows that in the treated vines in 9 cases out of 16 there was an increase in diameter at the graft union. Furthermore, it is possible to see a general tendency for an increase in diameter of the rootstock, especially with Lambrusco Grasparossa, although the greatest increase was in the Ruländer/41B combination.

Table 5

Influence of heat-treatment (30 min at 50 °C) on growth parameters of 16 grape cultivar/rootstock combinations

Einfluß der Wärmebehandlung (30 min bei 50 °C) auf die Wachstumparameter von 16 Kombinationen von Ertrags- und Unterlagenrebsorten

Combinations	Graft union* dia. (mm)		Trunk* dia. (mm)		Cv. canes (no.)		Cv. cane length (cm)	
	No heat	Heat	No heat	Heat	No heat	Heat	No heat	Heat
Albana								
420A	15.0	16.3	**7.9**	**9.0**	1.2	1.3	28.6	25.8
41B	16.8	16.1	8.9	9.2	1.3	1.1	**34.8**	**24.9**
5BB	17.6	16.4	9.5	9.1	1.3	1.1	35.1	28.5
1103P	14.8	15.9	8.3	9.5	1.2	1.4	28.9	30.2
Lambrusco G								
420A	15.9	15.8	8.9	9.1	1.4	1.6	23.6	30.0
41B	16.3	17.3	**8.6**	**9.7**	1.6	1.3	30.2	34.8
5BB	17.1	16.3	8.8	9.1	1.2	1.6	31.2	37.3
1103P	15.7	15.8	9.1	9.2	1.4	1.7	30.6	30.7
Ruländer								
420A	14.0	14.8	8.1	7.9	1.2	1.3	25.2	33.4
41B	14.0	15.7	**8.1**	**9.9**	1.1	1.2	25.9	30.8
5BB	15.7	16.6	8.6	9.0	1.5	1.4	37.8	37.5
1103P	**16.8**	**13.9**	8.4	7.8	1.6	1.4	**42.4**	**32.6**
Fortana								
420A	15.2	14.3	8.1	8.6	**1.2**	**1.6**	26.6	33.7
41B	**14.6**	**16.5**	9.1	8.8	**1.1**	**1.5**	**22.1**	**35.5**
5BB	**14.6**	**16.7**	9.6	9.9	1.4	1.7	29.4	41.6
1103P	14.9	14.3	8.1	8.9	**1.5**	**1.8**	**31.7**	**41.5**

* Significant at $P < 0.01$ (F-test).
Significant ($P < 0.05$; t-test) mean values of heat-treated and untreated plants, within each cultivar, are given in bold type.

The general tendency was confirmed for an increase in the number and length of canes after treatment and this was statistically significant in 9 cases, mainly concerning Fortana and regardless of the rootstock on which it was grafted. It was also observed that only 4 combinations out of 16 (Albana on 41B and 5BB and Ruländer on 5BB and 1103P) showed inferior growth in the treated as compared to the control vines.

U.S. experiments

The temperature of the water bath dropped to 48 °C when cuttings were first submersed, but stabilised at 50 °C within 3 min. The internal temperature of the cuttings reached 50 °C at all locations, even at the center of the bundles within 4 min. The temperature then remained stable throughout the experiment.

The hot-water treatment usually, but not always, reduced the level of AT3 in cuttings (Table 6). The majority of the untreated samples of cultivar and rootstock cuttings were naturally infected with AT3, however only 6 tumorigenic strains were isolated from both experiments over a 2-year period. The treatment adversely affected bud survival in a few cases, however, at least 57 % of the buds were viable for all cultivars and rootstocks. The treatment also stimulated increased callus formation in almost all cases.

Table 6

Heat-treatment of dormant grapevine cuttings (30 min at 50 °C) at Foster Nursery, Fredonia N.Y., U.S.A.

Wärmebehandlung dormanter Holzabschnitte (30 min bei 50 °C), Foster Nursery, Fredonia N.Y., U.S.A.

Cv. or rootstock	Infected/ indexed [1]		No. AT3 found		% Bud survival [2]		Callus formation [3]	
	No heat	Heat	No heat	Heat	No heat	Heat	No heat	Heat
Experiments in 1989:								
Niagara	6/20	0/20	0	0	80	75	1.7	2.1
Ravat	4/20	0/20	1	0	63	76	1.5	2.3
Seyval	2/20	0/20	0	0	72	81	1.2	2.2
Vidal	6/20	3/20	1	1	61	79	1.6	2.1
3309	3/20	3/20	0	0	76	80	0.9	1.2
Kober 5BB	13/20	6/20	0	0	86	57	2.1	2.2
SO4	8/20	4/20	0	0	75	69	2.3	2.7
Experiments in 1990:								
Niagara	8/21	0/21	0	0	73	56	0.8	1.4
Ravat	2/22	6/29	0	1	84	75	0.6	1.5
Seyval	0/25	0/25	0	0	88	87	1.8	1.2
Vidal	9/21	0/23	1	0	72	57	0.3	0.6
Kober 5BB	3/25	6/24	0	1	60	58	1.6	0.7

[1]) The treatments were performed on 15. 02. 1989 and 06. 03. 1990. Cuttings were indexed within 5 weeks of treatment as described in the text.
[2]) Percentage of buds (3–4 node cuttings) that grew.
[3]) Relative production of callus at basal end of cuttings. Score of 0–3 (0 = no callus, 3 = callus covering entire basal end).

Discussion

Severe recurrences of grapevine crown gall were noticed in many areas in Northern Italy following heavy frost damage in the winters 1984—1986 (BAZZI and BURR 1986). Today it is still possible to see diseased vines with old tumours at the crown and on the aerial parts. However, these do not often lead to the formation of new galls. For

the experiments carried out in Italy, canes were collected from cultivars in vineyards with a recent history of the disease, presuming that this would provide an abundant endophytic presence of AT3. Our results, however, showed a low level of systemic infestation, probably due to the mild temperatures over the last 3 winters, which were not conducive to new outbreaks of disease. For the U.S. experiments, 11 of 12 samples were found to be contaminated with AT3 and incidences ranged up to 65 %.

The failure to detect AT3 in some cultivars may also be attributed to the detection limits of the callus indexing method that was used (OPHEL *et al.* 1990) and/or the relative distribution of agrobacteria within the cuttings. Greater numbers of AT3 cells are found in the nodal regions of cuttings (STEFANI and BAZZI 1990) and, therefore, may also affect the results of indexing assays. However, there is always the risk of collecting latently infected material from apparently healthy mother plants, as in the case of rootstock 1103P.

Despite the low infection levels found, it was possible to confirm the potential usefulness of the hot-water treatment as reported in other experiments (BURR *et al.* 1989; OPHEL *et al.* 1990). In most cases, 30 min was sufficient to eradicate the pathogen from non-grafted cuttings. The positive effect of the 30 min hot-water treatment was subsequently confirmed by analyzing the callus of the 16 graft combinations after the forcing period, which always gave negative results. These data indicate the potential usefulness of this simple, economic and ecologically sound method of managing crown gall, even if failure to detect AT3 in the treated materials does not completely exclude the presence of a small number of endophytic heat-tolerant agrobacteria. This possibility has already been pointed out in previous papers (BURR *et al.* 1989; OPHEL *et al.* 1990).

Our results also clearly demonstrate that a 30 min treatment at 50 °C is not always effective in eliminating all of the AT3. Similar results have recently been obtained in Australia (OPHEL *et al.* 1990). Failure of the treatment was not the result of fluctuating temperatures or failure of internal grapevine tissues to quickly reach 50 °C, since even when large numbers of cuttings were simultaneously treated the internal tissue reached 50 °C within 4—5 min. These results agree with previous experiments that were done with single cuttings (BURR *et al.* 1989; OPHEL *et al.* 1990). Therefore, to improve the treatment, a higher temperature and/or a longer treatment period may be effective. Current investigations are underway to assess differences between AT3 with regard to heat sensitivity and various other aspects that may influence the heat sensitivity of strains as well as the viability of cuttings. A recent report has demonstrated that during the dormant season the treatment has a very significant effect on bud survival (WAMPLE *et al.* 1991). The effect of hot-water treatment on growth parameters varied with the scion/rootstock combinations. After the period in the field nursery, there was no sign of hypogeal and/or epigeal tumours which could in any way influence growth.

On the basis of an evaluation of growth parameters, it is possible to state that heat-therapy did not generally have a detrimental effect on vine vitality, on the contrary, in many cases, an improved growth was found. This can possibly be attributed to an elution of inhibitors which has been reported to occur in cuttings after immersion in water (CALABRESE 1965; CRISTOFERI *et al.* 1975).

Average mortality of treated vines was slightly less than controls; this was particularly marked with all the Fortana combinations. The results obtained with this cultivar and with Lambrusco Grasparossa were particularly interesting since they indicate a growth-promoting effect of heat-treatment and an improved graft-take, although the latter was only found on 41B and 1103P.

On the basis of the results of these experiments, heat-treatment, which to a certain extent is effective in eradicating AT3, can be recommended as a routine nursery practice to reduce the risks of latent infections.

Summary

Hot-water treatment (50 °C for 20—30 min) was carried out to confirm its efficacy in eradicating *Agrobacterium tumefaciens* biovar 3 (AT3) in symptomless grape cuttings.

After the forcing period, analyses of callus from cuttings of grape cvs Albana, Lambrusco Grasparossa, Ruländer and Fortana, and from their graft combinations with the rootstocks 420A, 41B, 5BB and 1103P, revealed the low infection level in the grape material used. Dormant scion and rootstock cuttings treated identically in the U.S. gave similar results. Despite this, it was possible to confirm the efficacy of thermotherapy in eradicating the pathogen.

An assessment was also made of the effect of treatment on growth parameters of grafted vines in the greenhouse and after 8 months in a field nursery. The effect of hot-water treatment on the vitality and growth of vines varied with the different scion-rootstock combinations. Treatment did not generally have detrimental effects on vitality; there were some negative effects on graft-take. The number and length of canes, as well as the diameter of the trunks, increased in most instances.

The treatments and times usually did not affect bud survival and, in most cases, increased the level of callus formation at the base of cuttings.

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