Nachrichtenbl. Deut. Pflanzenschutzd., 44 (4), S. 73–79, 1992, ISSN 0027-7479.

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Testing the Efficiency of Disinfectants for Control of Virus – Results achieved in disinfection experiments and proposal for proofing guide lines

Prüfung der Wirkung von Desinfektionsmitteln zur Kontrolle von Viren – Ergebnisse von Desinfektionsversuchen und Vorschläge für Prüfungsrichtlinien

Von N. Paludan

Summary

To avoid contamination and infection of pathogens, plants have to be grown under high hygienic conditions. In order to achieve this, disinfectants with high efficiency are needed. While lines of direction have been made for proofing disinfectants in ornamentals concerning bacteria and fungus (Anon. 1989) no procedure exists for viruses.

To achieve reproducible results experiments concerning the efficiency of different disinfectants for control of viruses were started at the Research Centre for Plant Protection in Lyngby in 1988 as described in this paper. The following methods were used:

- suspension test with short time treatment at a high infection rate to show the ability of disinfectants to virus inactivation
- suspension test with long time treatment at a lower infection rate with and without organic substances added to show the efficiency of disinfectants to act as surface disinfectants
- knife decontamination test to demonstrate the efficiency of disinfectants to inactivate existing virus on implements.

The efficiency of a disinfectant was dependent on the virus species present, the concentration of the disinfectant used and the period of treatment.

Increasing the concentration of the disinfectant and prolonging the period of treatment both increased the virus inactivation substantially.

Tomato mosaic virus turned out to be one of the virus most difficult to inactivate and was therefore very suitable as a test virus.

Addition of peat to the disinfectant did not cause any or only a very slight reduction of the efficiency of the disinfectant.

Elimination of virus on implements was achieved after 5 to 10 minutes of treatment; a shorter period of treatment or only dipping did not eliminate the virus.

Zusammenfassung

Um Kontaminationen und Infektionen durch Pathogene zu entgehen, müssen Pflanzen unter sehr guten hygienischen Bedingungen angebaut werden. Richtlinien zur Prüfung von Desinfektionsmitteln gegenüber Bakterien und Pilzen (Anon. 1989) sind ausgearbeitet worden. Es gibt aber keine Richtlinien für Viren.

Um reproduzierbare Ergebnisse zu erhalten, wurden im Pflanzenschutzzentrum in Lyngby im Jahre 1988 Versuche zur Untersuchung der Wirkung von verschiedenen Desinfektionsmitteln gegenüber Viren angefangen. Die Ergebnisse werden in diesem Artikel beschrieben. Folgende Methoden wurden angewandt:

- Suspensionstest mit kurzer Einwirkzeit und hohem Infektionsniveau, um die Fähigkeit des Mittels zur Virusinaktivierung zu prüfen.
- Suspensionstest mit langer Einwirkzeit und niedrigem Infektionsniveau mit und ohne Beimischung organischer Stoffe um die Wirkung als Oberflächendesinfektionsmittel zu prüfen.
- Messerdekontaminationstest um die Möglichkeit zur Inaktivierung des existierenden Virus auf den Werkzeugen zu prüfen.

Die Wirkung der Desinfektionsmittel war von der Virusart, der angewandten Konzentration und der Einwirkzeit abhängig.

Höhere Konzentrationen der Desinfektionsmittel und längere Einwirkzeit verursachen eine wesentliche Steigerung der Virusaktivierung.

Die Versuche zeigten, daß Tomatenmosaikvirus eines der am schwierigsten zu bekämpfenden Viren ist und deshalb als Testvirus sehr geeignet ist.

Die Wirkung der Desinfektionsmittel wurde gar nicht oder nur sehr wenig durch die Beimischung von Torf beeinflußt.

Eine Elimination des Virus auf den Werkzeugen wurde nach einer Einwirkzeit von 5 bis 10 Minuten erhalten; eine kürzere Einwirkzeit oder nur ein Eintauchen konnte das Virus nicht eliminieren.

During cultivation and propagation of horticultural plants there is always a risk of infection and spread of pathogens such as viruses.

The infection rate amongst viruses varies considerably, some of them being both highly infectious and stable and therefore easily transmitted. The transmission occurs especially during handling and cutting of plant material and from contaminated surfaces.

Earlier work has shown that both chemicals and organic matters can inactivate virus and control virus transmission (Thornberry, 1967; Rast, 1977, 1987; Paludan, 1988, 1990, 1991).

In order to obtain more information about efficiency of existing disinfectants, experiments were carried out during the years 1988–91 at the Research Centre for Plant Protection in Lyngby. The experience achieved and results from different methods of proofing inspired by and partly based on the German guidelines, (Anon. 1989, Böhmer, 1990) have furthermore formed the basis of the proposed guidelines for proofing of disinfectants against viruses.

Method

Viruses

The disinfectants were mainly tested for efficiency for control of tomato mosaic virus (TomMV), but five other viruses were also investigated as follows:

– Cucumber green mottle mosaic tobamovirus (CGMMV), originating from a Danish cucumber culture DK-No. 1:1:2–1981.
– Cucumber mosaic cucumovirus (CMV) serotype D-song, originating from H. LECOQ, Montfavet, France, DK-No. 2:10:3–1985.
– Cucumber necrosis tombusvirus (CNV), originating from G. ADAM, Braunschweig, Germany, received 1991.
– Melon necrotic spot carmovirus (MNSV), originating from L. BOS, Wageningen, The Netherlands as No. Cu15–1990.
– Pelargonium flower break carmovirus (PFBV), originating from a Dutch isolate as DK-No. 119, 7–1989.
– Tomato mosaic tobamovirus (TomMV) originating from a Danish Pepper culture as DK-No. 87, 73–1981.

Disinfectants

The following disinfectants were included in the investigations:
– Brown soap (40 per cent vegetable oils pre-soaped with potassium hydroxide added calcium carbonate).
– Technical trisodium phosphate (Na_3, PO_4, 12 H_2O).
– Detergent "Lissapol" (92 per cent alkyl phenol ethylene oxide condensate, 3 per cent isobutanol).
– "Korsolin" (3.8 per cent formaldehyde, 8 per cent glutaraldehyde).
Ferrosan A/S. Denmark.
– Venno Terra Spray (organic acids).
Venno Chemie GmbH, Norderstedt, Germany.
– Venno Cycla 2 (organic acids).
Venno Chemie GmbH, Norderstedt, Germany.
– Skim milk powder.
Monster "Milchpulver" 11/12 1989, The Netherlands.
– Skim milk (max 0.3 per cent milk fat).
Irma Store, Denmark.

Disinfectants techniques

The disinfectants were tested both by suspension experiments and by knife decontamination experiments.

Suspension experiments

The suspension experiments are conducted in the laboratory and the tests comprise different concentrations of the disinfectants and period of treatment as well as in some of the tests the effect of exposing the disinfectants to organic substance. The experiments comprise:

Short time treatment without organic substance at a high infection rate to show the ability of disinfectants to inactivate virus

TomMV infected tobacco plants (*Nicotiana tabacum* 'Samsun') are used as inoculum. The plant sap is squeezed out and equal part of 0.03 M phosphate buffer pH 7.7 is added and the mixture centrifuged 6000 rpm for 10 minutes. The supernatant is used and the infectivity of the virus is assessed by determination of the dilution end point.

The following treatments are performed:
– 0.5 ml virus suspension is added to 4.5 ml of the disinfectant (infection rate 1:10).
– The disinfectant is tested in the dilutions 1:4, 1:2 and 1:1 of the concentration recommended or an increasing concentration from 5 to 25 per cent.
– The pH of the actual concentration of the disinfectant used is rated.
– The effect of the disinfectant is assessed after ½, 5 and 10 minutes.

As a control treatment the disinfectant is exchanged with water to assess the infectivity of the treatments (Fig. 1). To assess a level of efficiency a treatment with brown soap (10 per cent) as a standard disinfectant is performed. The virus suspension and disinfectant mixture is inoculated to detached leaves of the tobacco plant *Nicotiana tabacum* 'Xanthi'. The leaves are powdered with carborundum No. 400 before the inoculation and rinsed with water after the inoculation. The leaves are placed in a humid chamber at 20 °C and 16 h light. The registration of symptoms is carried out after 1 week by counting local lesions (Fig. 2) calculated as a mean of 3 replicates.

Comprising other viruses, whole specific indicator plants grown in greenhouse reacting with local lesions, have been used as follows: *Chenopodium amaranticolor* for CGMMV and CNV, *Chenopodium quinoa* for CMV and PFBV and *Cucumis sativus* for MNSV.

Long time treatment with and without organic substance at a low infection rate to show the efficiency of disinfectant acting as surface disinfectant

The investigations are performed as described for the short time treatment but with the following changes:
– 0.1 ml of virus suspension is added to 10 ml of the disinfectant (infection rate 1:100).
The disinfectant ist tested in concentration according to request, or the most efficient concentration found.
– The effect of the disinfectant is assessed after 30, 60 and 240 minutes.
– The disinfectant is tested with and without organic substance added.

Using organic substance 0.1 g of unfertilized, air dried, white peat (grain size $\leq$ 2 mm) is added to 10 ml of the disinfectant with a 30 minutes period of incubation. The peat remains in the solution during the further treatments.

Knife decontamination experiments

The knife contamination experiments are conducted in the glasshouse and the tests of a disinfectant comprise different concentrations and periods of treatment to demonstrate the efficiency to inactivate existing virus on the knife.

TomMV-infected *N. tabacum* 'Samsun' plants are used as infector. The following treatments are performed:
– With a flamed but cooled scalpel a piece of the infected tobacco leaf is cut off.
– The scalpel is then without prior drying dipped into 10 ml of the disinfectant for a period of 1, 5 and 10 minutes respectively.
– The disinfectant is tested in the concentration recommended, or in the most efficient concentration found.
– After treatment 3 cuts are made with the scalpel into the stem of a healthy tobacco plant *(Nicotiana benthamiana)* (Fig. 3).

For the control of the virus infectivity a disinfected scalpel (no dipping) is used. Controls with water (Fig. 4) and brown soap treatment are performed as for the short time treatment.

Flaming is carried out between replicates and experimental plots.

Registration of symptoms on the plant is performed 14 days after the inoculation (Fig. 5). Infected plants die off. For each experimental plot 3 replicates are performed, each comprising 1 plant.

Fig. 1. Detached leaves of *Nicotiana tabacum* 'Xanthi' from long time suspension experiments with the disinfectant brown soap in 10 % and 1 % respectively showing high and lack of efficiency to control TomMV.

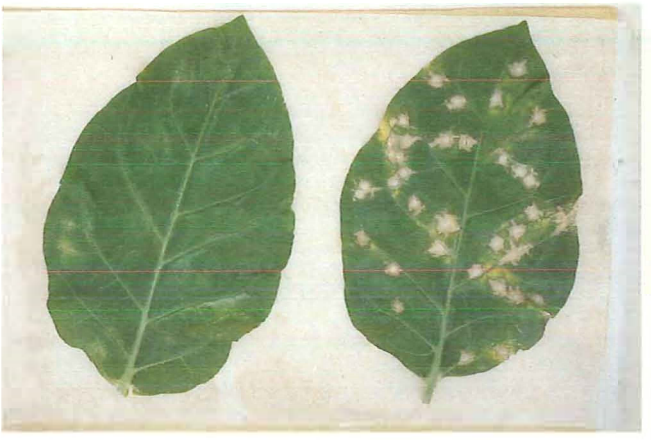

Fig. 2. Preliminary suspension trials with TomMV and *Nicotiana benthamiana* comprising: Water control (1st row from left), trisodium phosphate 10 % added Lissapol 1 % (2nd and 3rd row) and brown soap 1 % (4th and 5th row) for periods of 5 and 10 minutes respectively.

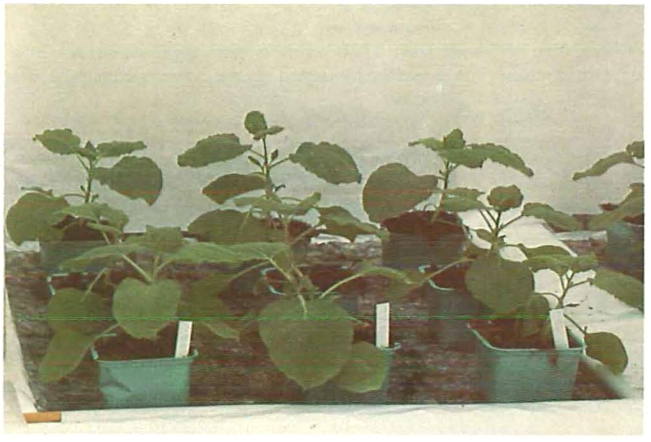

Fig. 3. Knife decontamination trials with TomMV and *Nicotiana benthamiana*.
Scalpel dipped in water for periods of 1, 5 and 10 minutes from left to right, respectively.

Fig. 4. Knife decontamination trials with TomMV and *Nicotiana benthamiana*. Scalpel dipped in trisodium phosphate 10 % added "Lissapol" 1 % for periods of 1, 5 and 10 minutes from left to right, respectively.

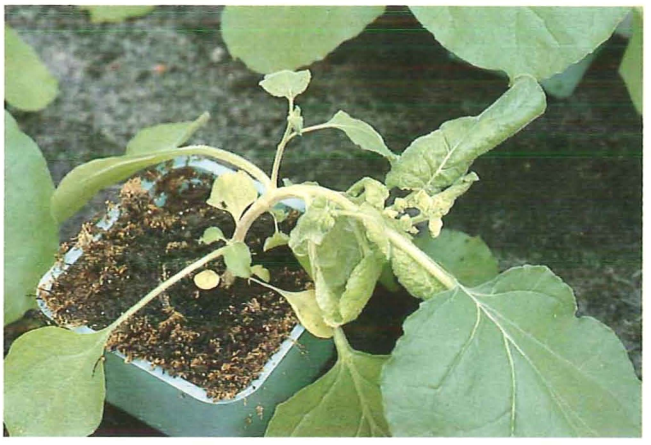

Fig. 5. Systemic reaction of TomMV in *Nicotiana benthamiana* after 10 days of the infection.

Results

Suspension investigations

Short time treatment without organic substance added at a high infection rate

Table 1. Suspension investigations comprising the efficiency of different disinfectants to control tomato mosic virus[1]) using indicator leaves.
Tab. 1. Suspensionstest: Die Wirkung von verschiedenen Desinfektonsmitteln gegenüber Tomatenmosaikvirus[1]) unter Verwendung von Indikatorblättern.

Disinfectant Desinfektions-mittel	Concentration of disinfectant (absolute) Konzentration des Desinfektionsmittels (absolut)	pH value pH-Wert	No. of local lesions[2]) after treatment in minutes Anzahl Lokalläsionen[2]) nach Behandlung in Minuten		
	%		½	5	10
Brown soap Schmierseife	5	11.4	103	19	45
	10	11.8	15	20	7
	20	12.1	10	0.3	1
	25	12.2	5	0	0.3
Trisodium phosphate + Lissapol 1%	5	12.1	100	6	2
	10[3])	12.1	150	4	5
	20[3])	12.0	400	150	68
	25	12.0	500	200	200
	38	11.9			
Skim milk powder Magermilch-pulver	5	6.9	193	167	157
	10	6.7	120	150	127
	20	6.7	72	55	58
	25	6.9	111	98	162
Skim milk Magermilch	25	6.8	60	30	32
	50	6.8	34	29	21
	100	6.7	4	6	3
"Venno Terra Spray"	25	2.6	150	7	35
	50	2.5	7	4	2
	100	2.6	9	0	0
"Venno Terra Man"	25	2.5	17	1	–[4])
	50	2.7	20	5	–
	100	3.1	3	2	–
Water control for all exp. Wasserkontrolle für alle Vers.	0	6.2	500	500	500

[1]) Dilution end point exceeds 10^{-6}.
Der Verdünnungsendpunkt war höher als 10^{-6}.
[2]) Average of 3 replicates. High nunbers only approximately.
Durchschnitt von 3 Wiederholungen. Hohe Zahlen nur angenähert.
[3]) Average of 6 replicates.
Durchschnitt von 6 Wiederholungen.
[4]) Test not performed the disinfectant being used for hand disinfection only.
Die Prüfung nicht durchgeführt; das Desinfektionsmittel wird nur für Handdesinfektion angewandt.

Long time treatment with and without organic substance added at a low infection rate

Table 2. Suspension investigations of the efficiency of different disinfectants to control tomato mosaic virus[1]) using indicator leaves.
Tab. 2. Suspensionstests: Die Wirkung von verschiedenen Desinfektonsmitteln gegenüber Tomatenmosaikvirus[1]) unter Verwendung von Indikatorblättern.

Disinfectant Desinfektionsmittel	Concentrat. of disinfect. (absolute) Konzentration des Desinfektionsmittels (absolut)	No. of local lesions[2]) after treatment in minutes Anzahl lokaler Schäden[2]) nach Behandlung in Minuten					
		30		60		240	
	%	Without peat Ohne Torf	With peat Mit Torf	Without peat Ohne Torf	With peat Mit Torf	Without peat Ohne Torf	With peat Mit Torf
Brown soap Schmierseife	1	38	81	48	85	10	67
	10	1	0	0	0.3	0	0
Trisodium phosphate[3])	1	0	0	0	0	0	0
	10	0	0	0	0	0	0
Skim milk Magermilch	1	500	500	500	500	500	500
	100	2	2	2	2	3	8
"Venno Terra Spray"	1	500	500	200	500	350	500
	100	0	0	0	0	0	0
"Venno Cycla 2"[4])	1	0.3	0.7	0	0.3	0	0
	10	0	0	0	0	0	0
Water control[5]) Wasserkontrolle[5])	0	500	500	500	500	500	500

[1]) Dilution end point exceeds 10^{-6})
Der Verdünnungsendpunkt war höher als 10^{-6})
[2]) Average of 3 replicates. High numbers only approximately.
Durchschnitt von 3 Wiederholungen. Hohe Zahlen nur angenähert.
[3]) The detergent "Lissapol" added in 1 per cent.
Das Detergens "Lissapol" zu 1 Prozent beigemischt
[4]) pH value 1%: 10%: 2.4
pH-Wert 1%: 2,5
[5]) Control for all experiments.
Kontrolle für alle Versuche.

Long time treatment with and without organic substance added at a low infection rate

Table 3. Suspension investigations of the efficiency of the disinfectant "Venno Cycla 2" to control different viruses using local lesions hosts.
Tab. 3. Suspensionstests: Die Wirkung des Desinfektionsmittels "Venno Cycla 2" gegenüber verschiedenen Viren unter Verwendung von Lokalläsionswirten.

Virus Virus	DEP[1]) reciprocal DEP[1]) reziprok	Concentr. of disinfect. (absolute) Konzentration des Desinfektionsmittels (absolut)	No. of local lesions[2]) after treatment in minutes Anzahl Lokalläsionen[2]) nach Behandlung in Minuten					
			30		60		240	
		%	Without peat Ohne Torf	With peat Mit Torf	Without peat Ohne Torf	With peat Mit Torf	Without peat Ohne Torf	With peat Mit Torf
CGMMV	−3	1	0	0	0	0	0	0
		Standart test[3])	0	0.3	0	0	0	0
		Water Control Wasserkontrolle	20	10	23	11	15	3
CMV	−3	1	0	0	0	0	0	0
		Water Control Wasserkontrolle	4	3	2	2	2	0.3
CNV	−6	1	2	6	10	27	5	0.3
		Standart test[3])	0.3	0.3	0.3	0	0	0.3
		Water Control Wasserkontrolle	500	500	500	500	500	500
MNSV	−4	1	0.3	0	0	1	0	0.3
		Water Control Wasserkontrolle	29	34	24	34	36	22
PFBV	−6	1	0	0	0	0	0	0
		Water Control Wasserkontrolle	80	55	70	60	60	55
TomMV	−6	1	0.3	0.7	0	0.3	0	0
		Standart test[3])	1	0	0	0.3	0	0
		Water Control Wasserkontrolle	500	500	500	500	500	500

[1]) Infectivity determined by dilution end point of the virus.
Infektionsfähigkeit, durch Verdünnungsendpunkt des Virus bestimmt.
[2]) Average of 3 replicates. High numbers only approximately.
Durchschnitt von 3 Wiederholungen. Hohe Zahlen nur angenähert.
[3]) Efficiency test with the standard disinfectant brown soap in 10 per cent.
Prüfung der Wirkung des Standarddesinfektionsmittels Schmierseife in 10prozentiger Lösung.

Knife decontamination investigations

Table 4. Knife decontamination investigations comprising the efficiency of different disinfectants to contro tomato mosaic virus using indicator plants.
Tab. 4. Messerdekontaminationstests: Die Wirkung von verschiedenen Desinfektionsmitteln gegenüber Tomatenmosaikvirus utner Verwendung von Indikatorpflanzen.

Disinfectant Desinfektionsmittel	Concentration of disinfectant (absolute) Konzentration des Desinfektionsmittels (absolut)	No. of infected plants compared to No. of treated plants after time of treatment in minutes. Anzahl infizierter Pflanzen im Vergleich zur Zahl der behandelten Pflanzen nach Behandlung in Minuten			
	%	Dipping Eintauchen	1	5	10
Brown soap Schmierseife	10	9/12	12/15	0/15	2/15
Trisodium phosphate[1])	10	11/12	9/15	0/15	0/15
	38	6/12	2/12	2/12	4/12
"Lissapol"	1	4/12	10/12	10/12	6/12
Skim milk powder Magermilchpulver	10	12/12	10/12	7/12	2/12
Skim milk Magermilch	20	4/12	8/12	5/12	1/12
"Korsolin" (pH: 4.2)	5	11/12	7/15	3/15	1/15
"Venno Terra Spray"	100	7/12	7/15	2/15	0/15
Water control[2]) Wasserkontrolle[2])	0	11/12	16/18	6/18	6/18
Virus control (untreated knife)					5/8

[1]) The detergent 'Lissapol' added in 1 per cent.
Das Detergens 'Lissapol' zu 1% beigemischt.
[2]) Control for all experiments.
Kontrolle für alle Versuche.

Discussion

A reduction of virus concentration or an inactivation of virus was achieved by the use of disinfectants, their efficiency being correlated to the concentration and the period of treatment as earlier described (THORNBERRY, 1967; RAST, 1977; PALUDAN, 1988, 1990, 1991).

The short time suspension test at a high infection rate (1:10) has shown this very clearly comprising five of six tested disinfectants (Table 1). The only deviation was found with trisodium phosphate causing a decrease of the inactivation capacity at concentrations higher than 10 per cent. The same deviation occurred also in the knife decontamination test (Table 4). These results are in conflict with THORNBERRY (1967), where up to 38 per cent were recommended as an effective treatment. MARCUSSEN and MEYER-KAHSNITZ (1991) found no effect of 10 per cent concentration during less than 5 minutes treatment, where the present results show good effect of 5 and 10 minutes. This is possible agreeable results taken the two different test methods used in consideration based on systemic and local leasions respectively, the latter reflecting the results quantitatively.

The lack of inactivation capacity at higher concentrations may be explained by a changed dissociation-behavior of the molecules (personal information from Dr. JUTTA HÖFFER, Hamburg).

In virus inactivation tests with pepper and tomato seed infected with different TMV-strains, nearly complete virus elimination was achieved using trisodium phosphate in 10 per cent concentration during periods from 15 minutes up to 2 hours (RAST, 1987; PALUDAN, 1988).

The skim milk powder showed an insufficient effect, irrespective of time and concentration used. Contrary full skim milk (100 per cent) showed high efficiency at all periods of treatment. This result did not correspond with the one described by RAST (1977), where 20 per cent was an effective concentration.

The short time suspension test performed with 0.5, 5 and 10 minutes (Table 1) has indicated that this test method is sensitive enough to select the usable disinfectant and furthermore to find both the optimal concentration and the needed time of treatment. The method also showed a high degree of reproducibility, where several repetitions gave consistent results. This could be a result of the performed centrifugation of the crude plant sap where organic matter is removed. The removal of organic matter will at the same time help the disinfectant to act, being more effective compared to natural conditions. However to be able to compare the efficiency of different disinfectants, it is most important to use a reliable and reproducible method.

The long time treatment at a lower infection rate (1:100) (Table 2) including the same virus, disinfectants and concentrations show continuous, corresponding results as for the short time treatment. All the tested disinfectants showed very high efficiency to control TomMV when used in the right concentrations. The trisodium phosphate completely eliminated the TomMV both of 1 and 10 per cent, which is in contrast to comparable results of MARCUSSEN and MEYER-KAHSNITZ (1991) using 5 per cent concentration during 2 hours.

Using the disinfectants 'Venno cycla 2' to control six different viruses (Table 3), high efficiency was achieved, also at one per cent concentration. This nearly correspond with MARCUSSEN and MEYER-KAHSNITZ (1991) results, but here virus control was achieved already after 15 minutes of treatment contrary 60 minutes. Cucumber necrosis virus turned out to be more difficult to inactivate than TomMV. Nevertheless both viruses are difficult to eliminate and therefore usable as parameters in tests of disinfectants.

The brown soap used as a standard disinfectant at 10 per cent concentration turned out to be equally or more effective. Brown soap was chosen as standard, because it is generally used for cleaning and disinfection in order to control virus.

As opposed to this no change in the efficiency was found either by the prolonged treatment from 30 to 240 minutes nor by pretreatment of the disinfectants with peat. Only deviation with peat was achieved with brown soap at insufficient low concentration.

These results are in contrast with those found for Venno Cycla 2 with the fungus *Fusarium oxysporum* being sensitive for both the period of treatment and for the addition of peat (BÖHMER, 1990).

Furthermore, no new evidence was found of more constant results by the use of the lower infection rate (1:100 compared with 1:10).

The conclusion to be drawn from these results could be to omit the long time treatment at a low infection rate and change the periods of treatment with the short time treatment at a high infection rate from 0.5, 5 and 10 minutes to 1, 5, 15 and 30 minutes. This would probably expose the efficiency of the disinfectant involved in a sufficient way.

The knife decontamination test has clearly demonstrated that the efficiency of the disinfectants are depending on the time of treatment, five minutes being the shortest sufficient.

All the tested disinfectants showed high efficiency towards TomMV used in the right concentration during 10 minutes.

This test method is important to demonstrate the efficiency the disinfectant in inactivating existing virus on implements. Time of treatment is suggested to be changed to 1, 5 and 15 minutes in order to get more clear differences between the results of the treatments.

Guidelines proposed for proofing of disinfectants for control of virus

The disinfectant is tested for efficiency to control tomato mosaic virus (TomMV) both by suspension experiments and by knife decontamination experiments.

Suspension experiments

The suspension experiments are conducted in the laboratory and the tests of the disinfectant comprise different concentrations and periods of treatment to show the ability to inactivate virus at a high infection rate:

TomMV infected tobacco plants (*Nicotiana tabacum* 'Samsun') are used as inoculum. The plant sap is squeezed out and equal part of 0.03 M phosphate buffer pH 7.7 is added and the mixture centrifuged 6000 rpm for 10 minutes. The supernatant is used and the standardization of the virus concentration is done by dilution end point of infectivity.

- 0.5 ml virus suspension is added to 4.5 ml of the disinfectant (infection rate 1:10).
- The disinfectant is tested in the dilutions 1:4, 1:2 and 1:1 of the concentration recommended.
- The effect of the disinfectant is assessed after 1, 5, 15 and 30 minutes.

As a control treatment the disinfectant is exchanged with water to assess the infectivity of the treatments. To assess a level of efficiency a treatment with brown soap (10 per cent) is performed as a standard disinfectant. The virus suspension

and disinfectant mixture is inoculated to detached leaves of the tobacco plant *Nicotiana tabacum* 'Xanthi'. The leaves are powdered with carborundum No. 400 before the inoculation and rinsed with water after the inoculation. The leaves are placed in a humid chamber at 20 °C and 16 h light. The registration of symptoms is carried out after 1 week by counting local lesions calculated as a mean of 3 replicates.

Knife decontamination experiments

The knife decontamination experiments are conducted in the glasshouse and the tests of the disinfectant comprise different concentrations and periods of treatment to demonstrate the efficiency to inactivate existing virus on the knife.

TomMV-infected *N. tabacum* 'Samsun' plants are used as infector. The following treatments are performed:
- With a flamed but cooled scalpel a piece of the infected tobacco leaf is cut off
- The scalpel is then without prior drying dipped into 10 ml of the disinfectant for a period of 1, 5 and 15 minutes respectively.
- The disinfectant is tested in the concentration recommended, or the most efficient concentration found.
- After treatment 3 cuts are made with the scalpel into the stem of a healthy tobacco plant *(Nicotiana benthamiana)*.

For the control of the virus infectivity an undisinfected scalpel (no dipping) is used. Control with water and brown soap treatment are performed as described for the suspension experiments.

Flaming is carried out between replicates and experimental plots.

Registration of symptoms on the plant is performed 14 days after the inoculation. Infected plants die off. For each experimental plot 3 replicates are performed, each comprising 1 plant.

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Nachrichtenbl. Deut. Pflanzenschutzd., **44** (4), S. 79–86, 1992, ISSN 0027-7479.

Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau, Weinsberg

Optimierung von Rebschutzversuchen nach biometrischen Gesichtspunkten, I. Versuche mit Botrytis cinerea Pers.

Optimization of viticultural field trials based on biometrical view, I. Experiences on Botrytis cinerea Pers.

Von G. J. Feurer und W. K. Kast

Zusammenfassung

Ziel der Arbeit war es, durch Anwendung biometrischer Verfahren Vorschläge für eine optimale Anlage, Durchführung und Auswertung von Versuchen in Weinbergen und Botrytis cinerea an Trauben zu erarbeiten. Ein Versuch wird dann als optimal betrachtet, wenn er die gewünschte Versuchsgenauigkeit mit einem minimalen Aufwand erreicht.

Die natürlichen Unterschiede im Befall von praxisüblich bewirtschafteten Weinbergen wurden mit Varianzkomponenten beschrieben. Erarbeitet wurden die Komponenten VKS für Stockunterschiede und VKC für kleinräumige Unterschiede. In daran anschließenden Blindversuchen wurde deren Einfluß auf die Fehlervarianz untersucht. Bei Botrytis cinerea sind erhebliche Befallsunterschiede innerhalb der Flächen vorhanden. Die Varianzkomponente zwischen Stöcken ist allerdings wesentlich größer als die Komponente für kleinräumige Unterschiede. Der Einfluß auf die Fehlervarianz wurde durch Simulationen von Blindversuchen ermittelt. Dabei wurden Daten aus Bonituren verwendet, in denen der Befall aller Trauben in 7–10 Rebreihen von je 40 Stöcken erfaßt worden war. Dabei wurde die Varianz bei Parzellengrößen von fünf bis 40 Stöcken in die Komponenten „zwischen Parzellen" VKZ und „innerhalb Parzellen" VKI zerlegt. Die Komponente VKZ nimmt in der Regel mit der Parzellengröße ab,