Factors Affecting Mechanical Spread of Exocortis Virus

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SPREAD OF CITRUS viruses in the field was long attributed to transmission by grafts, insects, or seed and not mechanical transmission (8). However, this picture was changed in 1967 by the demonstration that exocortis virus (CEV) can be transmitted as a contaminant on budding knives (4, 6). Other reports (1, 9, 13) followed, and contamination is now suspected of being responsible for the unexplained spread of CEV among greenhouse-grown and field-grown citrus plants (2, 5). It seems appropriate in these proceedings to review the research pertaining to mechanical transmission of CEV and to present some additional relevant and previously unpublished findings.

Inoculation Procedures

Mechanical transmission of CEV was first demonstrated by Weathers (11), who transmitted it from petunia to petunia after having transmitted it first from citron by means of dodder (*Cuscuta subinclusa* Dur. & Hilg.). Results in these early mechanical transmission tests were erratic. The first attempts to transmit the virus from citrus or petunia to citrus with conventional leaf inoculation techniques failed (11), but erratic success was later achieved (13). Garnsey and Jones (6) also failed to infect citron plants with CEV by leaf inoculation but obtained a very high percentage of infection by cutting citron stems with a knife contaminated by cutting infected plants. Our results from leaf inoculation remain erratic and unreliable in contrast to those of Fudl-Allah (3), who reported consistent 100 per cent transmission by leaf inoculation.

Inoculation procedures that involve cutting or crushing stem or leaf tissue of the indicator plant, so as to place CEV in direct contact with phloem tissue, consistently give a high percentage of infection with short incubation and are useful for making in vitro infectivity assays (1, 4, 9, 10, 13). A comparison of the efficiency of several inoculation procedures is presented in Table 1. Infection rates of 90 per cent or more were achieved in citron indicator plants by making 10 stem cuts with a contaminated knife and by crushing leaf midribs in the presence of a drop of inoculum. Infection occurred more readily when the inoculation wound was made in the presence of the virus than when a sterile wound was made and CEV subsequently applied.

In early tests, Garnsey and Jones (6) wrapped the site of inoculation with rubber tape. In the present tests, wrapping did not increase infection

but did reduce the incubation period (Table 1). Incidentally, wrapping helps to strengthen succulent stems and prevent breakage.

Similar results with wrapping have been obtained in inoculation trials with petunia, *Gynura aurantiaca,* and *G. sarmentosa.* In addition, inoculation by needle puncture has been effective with herbaceous hosts (10,

spread by contaminated hands as well (9).

The possibility that chewing insects could be vectors led to limited tests with grasshoppers and katydids. CEV was transmitted to one citron plant by the Eastern Lubber grasshopper (*Romalea microptera* Beauv.), but this result was not confirmed in subsequent tests.

TABLE 1. Relative efficiency of inoculation techniques for transmitting cev to Etrog citron plants (Arizona 861 selection)

Technique[a]	No. plants infected/inoculated	Incubation period (avg) (days)
10 cuts on stem with contaminated knife (wrapped)	9/10	49
10 cuts on stem with contaminated knife (not wrapped)	10/10	78
Inoculum applied to 10 cuts made with sterile knife	3/10	85
Leaf midribs crushed in presence of inoculum	9/10	85
Leaf inoculated, carborundum method	7/10	117
Noninoculated control	0/10	

a. Inoculum was a 1:9 dilution (w:v), in neutral phosphate buffer, of an extract of infected young bark filtered through a 5 μm in filter; it was applied dropwise to the cutting knife, to cuts in stem, or to a midrib before crushing it with forceps; self-adhesive rubber tape was used for wrapping; plants were randomized after inoculation.

12, 13). Stem and leaf vein tissue were more susceptible than roots (Table 2).

From the practical standpoint, wounds produced in normal field or nursery operations—such as budding, pruning, suckering, and cultivating—can provide sites for establishing exocortis virus infections. Contamination is not limited to cutting tools, and CEV may be

Virus Properties and Effect of Sterilants

The erratic transmission patterns obtained in early attempts to transmit CEV mechanically suggested that it was unstable in plant extracts (13). Yet attempts to decontaminate tools indicate that CEV is surprisingly stable.

Garnsey (4) reported that CEV

survived for 16 hours on a dry knife blade. Allen (1) found that the virus was infectious after 8 days on a dry blade. Infectivity of CEV in extracts made with neutral phosphate buffer was greatly diminished or completely lost after 2 hours. On the other hand, aqueous solutions of partially purified CEV retained their infectivity at room temperature for several months (10).

been ineffective. Garnsey and Jones (6) reported that exocortis virus was not inactivated on budding knives by brief exposure to 70 per cent ethyl alcohol, but it was inactivated by an aqueous solution containing 2 per cent formaldehyde and 2 per cent sodium hydroxide. The effectiveness of the latter solution was confirmed by Roistacher et al. (9), who reported that a dilute solution of house-

TABLE 2. SUSCEPTIBILITY OF STEM AND ROOT OF YOUNG, ROOTED
CUTTINGS OF CITRON (ARIZONA 861 SELECTION) TO
INFECTION BY CEV

Inoculation site	No. plants infected[a]		
	Knife drawn through infected stems	Knife drawn through infected roots	Sterile knife
Stem	5/5	5/5	0/5
Feeder roots	1/10	0/10	0/5

a. Inoculations were made by drawing a knife 10 times through a stem or feeder root of a test plant.

Semancik and Weathers (10) provided evidence that CEV exists as free RNA, i.e., without a protein coat. Sap extracts remained infectious when heated for 10 min at 80°C. Partially purified preparations of CEV remained infectious after boiling for 20 min. Roistacher et al. (9) found that briefly heating contaminated blades in the flame of a propane torch was ineffective, even though blade temperatures as high as 260°C were measured. Flaming knife blades dipped in alcohol was also ineffective. It appears that heat treatment is not a practical means for rapidly sterilizing tools contaminated with CEV.

A number of common chemical sterilants have been tested for their effect on exocortis virus. Many have

hold bleach (sodium hypochlorite) was also an effective sterilant—and claimed it preferable to the formaldehyde-sodium hydroxide mixture for practical reasons. These workers confirmed the ineffectiveness of ethyl alcohol as a sterilant and showed that solutions of Physohex (20 per cent), Lysol (1 per cent), Borax (10 per cent), and trisodium phosphate (2 per cent) were also ineffective for decontaminating tools (9).

Household detergents (2 per cent), trisodium phosphate (10 per cent), sodium hydroxide (2 per cent), dimethyl sulfoxide (10 per cent), and potassium permanganate (1 per cent) were ineffective in our tests. The sodium hydroxide and trisodium phosphate solutions

did reduce transmission somewhat and increased the incubation period required for symptoms to appear. Alkaline hydrolysis of the CEV–RNA was probably occurring, but too slowly to be effective in our tests. Sodium hypochlorite was completely effective at concentrations of 0.5 per cent and 0.25 per cent, and 90 per cent effective even at a concentration of 0.05 per cent.

Solutions containing at least 2 per cent formaldehyde can prevent transmission of CEV as a contaminant, but the effect of formaldehyde is apparently on the plant cells at the site of inoculation and not on the virus. When freshly contaminated knives were dipped in formaldehyde and used to make inoculation cuts while still moist, no infection occurred (10 plants). When the knife blades were allowed to dry after being dipped in formaldehyde and then used to make inoculation cuts, all 10 plants became infected. Blades dipped in a 0.5 per cent sodium hypochlorite solution and dried before use did not transmit CEV, indicating a direct effect on the virus.

Based on effectiveness, availability, and cost, sodium hypochlorite is the best sterilant for CEV among those tested. The inactivation process has not been studied. Sodium hypochlorite is a strong oxidizing agent, but this may not be the entire explanation, since a solution of potassium permanganate had no apparent effect. Unfortunately, sodium hypochlorite is corrosive to metals, bleaches clothing, and can irritate skin. Roistacher et al. (9) recommended dipping tools in a mixture of vinegar, water, and emulsifiable oil to prevent corrosion following treatment with sodium hypochlorite. This procedure, however, complicates the process of sterilizing tools in the field.

The need remains for an effective chemical with no undesirable side effects. Other halogenated compounds deserve testing. Moreover, the ability of the enzyme ribonuclease to inactivate CEV (10) suggests that it should be tested under field conditions.

Varietal Susceptibility

Orange and grapefruit seedlings were less susceptible to infection by knife-cut inoculation than citron, regardless of whether citron, orange, or grapefruit tissue was used as an inoculum source (4).

Investigation of the susceptibility of seedlings of various citrus varieties to mechanical inoculation revealed striking differences (Table 3). The low susceptibility of varieties such as Duncan grapefruit and Orlando tangelo may be useful for controlling spread of CEV by contamination in the field. For example, a resistant variety would be a logical choice to plant in areas where the risk of contamination is great. Further studies may detect a low susceptibility in other varieties.

Inoculum Source

The donor host is apparently less important than the receptor host for mechanically transmitting CEV be-

TABLE 3. Susceptibility of citrus plants to
inoculation with exocortis virus by
a contaminated knife[a]

Variety	No. plants inoculated	No.[b] infected
Etrog citron (Arizona 861)	20	20
Rangpur lime (3 selections)	30	30
Trifoliate orange	10	9
Morton citrange	20	18
Eureka lemon	30	24
Sour orange	20	9
Mexican lime	20	6
Pineapple sweet orange	85	12
Rough lemon	20	2
Duncan grapefruit	37	1
Orlando tangelo	10	0
Rusk citrange	20	0

a. A knife contaminated each time by passage through the stem of an infected citron plant was used to make 5–10 cuts in the stem of a test plant.
b. Symptomless plants were tested for CEV by graft-inoculation of citron plants.

tween closely related plants, but it is an important factor when transmission is attempted between unrelated plants. Citron is an excellent donor host for knife inoculations to citron and other citrus, but serves less well for inoculations to petunia and gynura. Transmission in either direction between citron and petunia or gynura was less than 30 per cent, whereas transmission between congeneric hosts exceeded 90 per cent. Grapefruit was a slightly poorer inoculum source than sweet orange or citron in the experiment reported by Garnsey (4), but these sources have not been thoroughly compared. Allen (1) transmitted exocortis virus to citron from Eureka and Ponderosa lemon, Navel and Valencia orange, Cecily grapefruit, and Citrumelo CPB 4475 by knife inoculation. Igwegbe (7) found calamondin to be a poor host for exocortis virus.

The relative titer of exocortis virus in different citrus hosts has not been ascertained, but apparently there is enough virus in most of them to contaminate knives sufficiently to transmit CEV to susceptible plants.

Virus Distribution in Different Tissues

Results from various inoculation tests (6, 13) suggested that CEV was not uniformly distributed in all tissues of infected plants. To test this observation more thoroughly, various tissues from infected Etrog citron plants were triturated in 0.05M neutral phosphate buffer, filtered through a 1.2 μ filter, and diluted with buffer to appropriate volumes. Assays were performed by dipping a knife blade into the diluted extracts and then making 10 cuts in stems of citron indicator plants. Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and sometimes 10^{-4} (w:v) were used.

Infections were produced by 10^{-2} dilutions of extracts of young and old feeder roots, young and old bark, young wood tissue, and leaf midribs of all ages. Extracts of young bark and leaf midrib tissue were usually infectious at a 10^{-3} dilution and often at a 10^{-4} dilution.

Extracts of leaf blade tissue were infectious at a 10^{-1} dilution but not at 10^{-2} dilution in a single test. Titer measurements vary according to the condition of the donor host, buffer system, and incubation period, and small differences are undetectable. Systemically infected citron plants have, however, substantial amounts of CEV in tissues that might logically serve as donor sources in the field.

Conclusion

Recognition of the fact that CEV can be easily transmitted as a contaminant and use of appropriate preventative measures are necessary to prevent spread of CEV by contamination. Nurserymen must use special caution to avoid contamination of their foundation trees and trees propagated for sale, because an unrecognized infection can be increased enormously by subsequent vegetative propagation. Controlling the spread of CEV in field plantings is more difficult than in the nursery, from the standpoint of decontaminating tools, but is perhaps less important practically. If trees on sensitive rootstocks attain considerable size before infection occurs, any subsequent stunting becomes less detrimental.

New plantings on exocortis-sensitive stock should be planned to avoid as many obvious sources of contamination as possible. Citrus varieties with low susceptibility to mechanical inoculation should be considered wherever the risk of contamination is high.

Knives and other wounding instruments that cut through phloem tissue of infected trees of most citrus varieties will become contaminated with CEV. Tools and hands can also be contaminated with sap from these wounds. Since CEV remains infectious for a long time, contaminated tools must be discarded or decontaminated.

Because CEV has a high resistance to heat, chemical treatment seems the best procedure for decontaminating CEV-contaminated tools. Despite some unpleasant side effects, a 0.25 to 0.5 per cent solution of sodium hypochlorite seems the best choice on the basis of current information.

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