Some Effects of Temperature on Symptom Appearance and Therapy of Citrus Impietratura Disease*

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Impietratura, a graft-transmissible citrus fruit disease (Ruggieri, 1961), affects sweet orange and grapefruit in many citrus-growing areas of the Mediterranean basin. In Israel, the overall number of infected trees does not seem to be high (Pappo *et al.*, 1967) but in some plantations up to 50 per cent of the trees are affected. The disease causes fruit drop, peel hardening, and albedo gumming. Budwood from impietratura-infected trees induced leaf-fleck symptoms on indicator seedlings (Bar-Joseph and Loebenstein, 1970) but, at present, the possibility cannot be ruled out that another virus of the psorosis group is present in all the impietratura trees examined. The intensity of infection including fruit drop, percentage of affected fruits, and symptom severity, varies among different varieties (Cartia and Catara, 1972), years, and individual trees (Pappo and Oren, 1974).

Scaramuzzi *et al.* (1968) noticed that the percentage of fruits with symptoms of impietratura is very low in fruits developing from the late (summer) bloom as compared with fruits from the normal spring bloom. Therefore, they raised the possibility that climatic factors may influence symptom expression of the disease.

This paper summarizes experiments carried out under temperature-controlled conditions in order to determine the effect of temperature on symptom appearance, transmissibility through seeds of Valencia, and virus inactivation by thermotherapy.

MATERIALS AND METHODS

Virus. Two impietratura isolates were used. The first isolate, Imp-BD, was from a 9-year-old Valencia tree at Bet Dagan. Most fruits of this tree were small, about a quarter of the normal size, and pear shaped with a large gum deposit under the calyx. Other fruits had either a few swollen areas or appeared normal. The second isolate, Imp-K, from a 12-year-old Valencia tree at Revadim, produced many small, malformed fruits with large gum deposits in the albedo.

Experimental temperature conditions for symptoms expression. Old clone Valencia on sour orange rootstock growing in 10-liter plastic bags were purchased from a commercial nursery and kept in a screenhouse. In the spring of 1970, ten chip-graft inoculations with Imp-BD were done on each 3-year-old plant, on branches 0.5-1 cm in diameter. At the end of March 1971, plants bearing flowering buds were transferred into growth rooms, which were plastic cabinets 190 cm high, in a glasshouse. The temperatures used were: (1) 22 ± 2°C, designated as the "cool" treatment; and (2) the "warm" treatment, with a minimum of 27°C and a maximum of 32 ± 1°C. To prevent heat shock, transfers between the cool and warm rooms included preconditioning for a week at 25 ± 2°C. Care, including irrigation and insect control, were given according to the special needs in each room.

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Each fruit was covered with a plastic net bag and bound to the mother tree. Examination of dropped fruits for symptoms was made every two days. Two inoculated trees were used for each treatment group.

Experiments on seed transmission. Seeds were collected from two trees growing continuously in the cold room, four trees grown in the cool and transferred to the warm room (Groups A and B, table 1), and from the Imp-BD source tree. The seeds were germinated in flats of vermiculite and peat (1:1) and transplanted into 3-liter pots containing soil, sand, and peat (2:1:1), with four plants in each pot. Seven-month-old seedlings were pruned and transferred to the cool room. Four buds from each Valencia seedling were grafted onto two Madam Vinous seedlings; each Madam Vinous received buds from 10 Valencia seedlings. After establishment of the grafts the plants were topped to force new growth and transferred to the cool room. Examination for leaf-flecking symptoms on the Valencia and Madam Vinous seedlings was made weekly. Two months after topping, two buds from each Madam Vinous were grafted onto 10-year-old grapefruit trees, each tree receiving buds from plants of a single seed source.

Thermotherapy. Eighteen-month-old Valencia and sour orange seedlings grown in 10-liter pails in a screenhouse were incoculated with Imp-BD and Imp-K isolates. After establishment of the grafts the seedlings were pruned and transferred to the cool room until leaf-flecking symptoms appeared. The plants were then transferred to a screenhouse and about five months following inoculation they were preconditioned for two weeks in the warm room. Thermotherapy was conducted in a plastic cabinet for five months at 38 ± 5°C. Virus inactivation, as evidenced by lack of ability to induce leaf-flecking symptoms and fruit gumming, was checked on Madam Vinous seedlings and on two 10-year-old grapefruit trees.

RESULTS

Effects of temperature on symptom appearance. Table 1 summarizes the experiments carried out during 1971-72. Trees kept continuously in either cool or warm conditions developed no symptoms. After the two trees of Group A, with fruits about 0.5-1.5 cm in diameter, were transferred from cool to warm conditions, 8 of 22 developed at least one gum deposit, usually under the calyx. Thirty of 42 fruits dropped within six weeks following the transfer of group A, as compared with 14 of 35 fruits in a noninoculated control tree. The dropped fruits did not have conspicuous gumming symptoms, although a few from the inoculated trees appeared to have a somewhat browner albedo than the controls. Later transfers, when the fruits were larger than 2.5 cm, resulted in a fruit drop at a rate similar to the control and no gum deposits were observed in the remaining fruits.

TABLE 1
EFFECT OF TEMPERATURE ON SYMPTOM APPEARANCE IN FRUITS OF IMPIETRATURA-INFECTED VALENCIA TREES

Treatment and group	Fruits with symptoms/fruits dropped	
	Tree 1	Tree 2
Continuously cool*	0/12	0/8
Cool then warm A†	6/12	2/10
Cool then warm B†	0/5	0/8
Cool then warm C†	0/8	0/11
Warm then cool D†	0/9	0/7
Continuously warm	0/9	0/8

*Cool = 22 ± 2°C; Warm = 27 (minimum) to 32 ± 1°C (maximum).

†A, transferred when fruits were 0.5-1.5 cm; transferred when fruits exceeded 2.5 cm; C, transferred when fruits exceeded 4.0 cm; D, transferred when fruits were 1.0-2.0 cm.

Seed transmission tests. Evidence of seed transmission of impietratura was sought among 58, 42, and 205 Valencia seedlings grown from fruits collected from trees kept continuously in the cool room, from transfers A and B (table 1)

and from the Imp-BD source tree, respectively. No leaf-flecking symptoms were observed on the test seedlings, nor on the Madam Vinous seedlings grafted with budwood from them. Furthermore, no fruit symptoms were noted after three years on three grapefruit trees grafted with budwood from these Madam Vinous plants.

Thermotherapy. Apparent inactivation of impietratura virus was achieved by growing two groups of plants, three Valencia and three sour orange seedlings, graft-inoculated with Imp-BD and Imp-K isolates, for five months in a cabinet kept at 38 ± 5°C. Both isolates appeared to be inactivated in the new growth of heat-treated seedlings since psorosis-like leaf symptoms were not induced following grafting on Madam Vinous, nor were fruit symptoms induced following grafting on grapefruit trees.

DISCUSSION

Impietratura affects the commercial value of infected trees by causing fruit drop and albedo gumming. The severity of infection varies on the same tree in different years (Pappo and Oren, 1974) and seasons (Scaramuzzi *et al.*, 1968). The appearance of symptoms among fruits transferred when 0.5-1.5 cm in diameter from 22 ± 2°C to 27-32°C, confirms the supposition of Scaramuzzi *et al.* (1968) that climatic factors influence symptom expression. The time of appearance of maximum gum reaction needs to be rechecked under more precisely defined conditions. It appears that symptoms of impietratura, like symptoms in some other virus-host combinations, such as exocortis bark scaling and stunting in *Poncirus trifoliata* (Weathers *et al.*, 1962), require special temperature conditions.

I suggest as a working hypothesis that impietratura fruit symptoms result from two events: virus replication in the early stages of fruit set at a cool temperature, and the gumming reaction later at a higher temperature. The timing of these events and optimum temperature conditions need to be studied but it seems that low temperature is required only in the early stage because symptoms did not appear when fruits larger than 2.5 cm were transferred from cool to warm conditions. A somewhat similar situation was reported when sesame plants were inoculated with Satsuma dwarf virus (Tanaka *et al.*, 1965). Infected plants showed severe symptoms when they were kept at 25°C for at least 8 hours after inoculation and then transferred to 36°C, but developed no symptoms when kept continuously at 34°C.

The exact nature of impietratura gumming is not understood, but recent experiments (Dr. A. Cohen, personal communication) showed that ethephon treatment caused a very intensive localized gumming on grapefruit and Shamouti branches. Thus, it may be that impietratura, like some other viruses (Lockhart and Semancik, 1970; Nakagaki *et al.*, 1970), induces ethylene production at some localized infected areas. We have made observations indicating that the systemic spread of impietratura virus is relatively limited. On two 30-year-old trees inoculated with Imp-BD, severe reactions appeared during the first season in fruits adjacent to the inoculated branches (1 cm in diameter) but no symptoms could be found even after four years in fruits of branches on the opposite side of the tree. It may be assumed that symptomless fruit on infected trees result either from temperature conditions unfavorable for symptom expression or that they have escaped infection due to limited systemic spread of the virus.

The results indicate that impietratura is not seed-transmitted in Valencia orange. Seed transmission is host and virus-strain specific (Shepherd, 1972); therefore, a final conclusion concerning this possibility has to await examination of all commercial rootstocks. Impietratura, like most citrus virus diseases (Calavan *et al.*, 1972; Nyland and Goheen, 1969), can be inactivated through thermotherapy. Such treatment might help both by freeing certain varieties, such as Navelina, which are almost 100 per cent infected with impietratura virus, and by clarifying the relationship between psorosis-like leaf symptoms and impietratura.

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