STUDIES ON THE MICROBIAL COLONIZATION OF SAPWOOD OF PRUNED APRICOT TREES

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Abstract

The colonization by microorganisms of apricot sapwood exposed by winter pruning was investigated. The number of microorganisms isolated increased rapidly, reaching a peak 12 days after pruning unsheltered trees and 15 days after pruning trees under a rain-proof shelter. Bacteria were the predominant colonizers on unsheltered trees and *Cladosporium herbarum* on sheltered trees. Unsheltered trees pruned later in winter showed a similar colonization pattern.

Copper oxychloride, applied to the cut surfaces of pruned stems, did not affect the composition of the microflora or pattern of colonization. Benomyl, applied as a post-pruning spray, had a marked fungicidal effect, and virtually no fungal colonies were isolated up to 18 days after pruning.

Out of 473 fungal and bacterial isolates only 33 showed any antagonistic activity to *Eutypa armeniacae*. Twenty-nine of these were species of *Fusarium*.

I. INTRODUCTION

The duration of susceptibility of apricot pruning wounds to infection by *Eutypa armeniacae* Hansf. & Carter, the causal organism of apricot "gummosis" or "dieback", was investigated by Carter and Moller (1970). They found that branches pruned in winter rapidly lost their susceptibility to infection, and postulated that this was owing to the competitive or inhibitory effects of microorganisms which colonized tissues exposed by pruning. Trees sheltered from the rain showed a lesser decline in susceptibility and it was considered that this could be attributed to a lack of water-dispersed microorganisms reaching the pruned surfaces. Carter (1971) stated that the incidence of apricot dieback in South Australia increased with the introduction of applications of copper fungicides to trees in autumn and suggested that this practice may deplete the trees of a surface microflora containing species capable of rapidly colonizing the vessels exposed at wound surfaces, thereby favouring the pathogen.

Moller and Carter (1970) found that benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate], applied immediately after pruning, greatly reduced the frequency of infections when *E. armeniacae* ascospores were inoculated onto wound surfaces 1 day later. However, their experiments did not reveal the duration of the protection which might be afforded by the fungicide. If protection against the

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pathogen by benomyl should prove short-lived, it would be important to know the duration of its effects against components of the microflora which might otherwise compete against *E. armeniacae* in the infection court.

This paper reports investigations of the colonization of sapwood exposed by winter pruning of apricot trees.

II. Materials and Methods

In 1971, experiments were conducted on 9-yr-old apricot trees cv. Moorpark at the Waite Institute. One row of 14 trees was sheltered from rain as described by Carter and Moller (1970). In 1972, experiments were conducted only on unsheltered 4-yr-old apricot trees of the same variety.

Selected leaders and laterals on experimental trees were tagged with coloured plastic ribbon just below a dormant bud prior to pruning.

(*a*) *Isolation of Microorganisms*

Tagged stems were cut off at 3-day intervals after pruning. The original wound surfaces were flamed for 1 s; preliminary tests had shown that this treatment was effective in killing organisms at the surface. Four holes were then bored, to a depth of 2 mm, through the flamed surface of the sapwood by means of a No. 4 dental burr (Ash Burrs, U.K.) attached to a hand drill. The wood particles from each of the borings were added to 15 ml of sterile molten Czapek–Dox agar (5% nutrient strength) and thoroughly mixed before pouring into sterile 9 cm Petri dishes. The agar was allowed to set and the plates then incubated at 25°C in the dark. The numbers of bacterial and fungal colonies that appeared on the plates were recorded after 1 week. Subcultures were made of the most frequent organisms and these were tested for antagonistic activity to *E. armeniacae*.

(*b*) *Antagonistic Activity*

The test organism was inoculated onto 5% Czapek–Dox agar at a distance of 4 cm from a 1-cm disk of agar containing a fresh culture of *E. armeniacae*. Plates were incubated in the dark for 1 week at 25°C, and then examined for inhibition of growth. Controls were the test organism, and *E. armeniacae* alone.

III. Experimental and Results

(*a*) *Colonization of Sapwood at Pruning Wounds within and outside a Shelter*

In all, 100 stems were tagged on five trees under the shelter and another 100 on seven trees outside the shelter. Each tree contained 10 or a multiple of 10 tagged stems. Stems were pruned immediately above a dormant bud on 21 June 1971 and 10 of the pruned stems (one or more per tree according to the total number on each tree) were removed from each treatment at 3, 6, 9, 12, 15, 18, and 30 days after pruning.

The number of colonies isolated increased with time, a peak being reached 12 days after pruning on trees outside the shelter and 15 days after pruning on trees under the shelter (Fig. 1). The population decreased 18 days after pruning on sheltered trees. With unsheltered trees, a similar decline in numbers occurred at 15 days but this was followed by a subsequent rise in the fungal population. Bacteria were the predominant colonizers on trees outside the shelter and fungi, mainly *Cladosporium herbarum* Link ex Fr., the predominant colonizers on trees under the shelter. *Aureobasidium pullulans* (de Bary) Arn. was present in both treatments with fewer numbers of *Alternaria* spp., *Penicillium* spp. and *Fusarium* spp. (Table 1).

(b) Colonization of Wounded Sapwood in the Presence or Absence of Copper Oxychloride

A second experiment, on unsheltered trees only, was begun 5 weeks later to determine whether a similar pattern of colonization of the sapwood occurred when the trees were pruned later in winter. In this experiment, the effect of copper oxychloride on colonization was also studied.

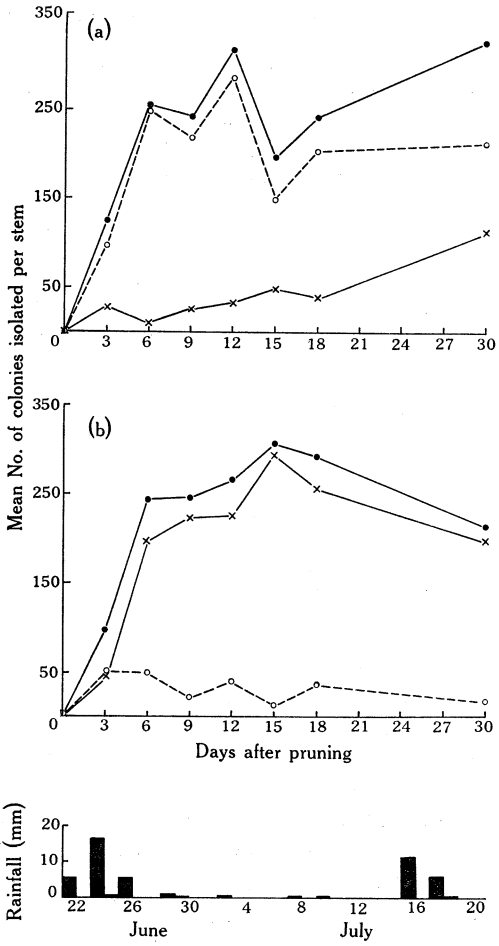


Fig. 1.—Colonization of sapwood of pruned apricot trees outside (*a*) and within (*b*) a rain-proof shelter. ● Total colonies. ○ Bacterial colonies. × Fungal colonies.

In all, 160 stems on seven trees in each of two adjacent rows were tagged prior to pruning. Each tree contained 8 or 16 tagged stems. Treatments were randomized, using single trees as plots. Tagged stems were pruned above a dormant bud on 27 July 1971 and a suspension of commercial copper oxychloride (12·5% a.i.; 0·75 g/200 ml water) was applied by means of a brush to the wounds on half the trees.

Ten tagged stems from each treatment were removed at 3, 6, 9, 12, 18, and 30 days after pruning and isolations of microorganisms made as previously described.

TABLE 1

BACTERIAL AND FUNGAL COLONIES ISOLATED FROM SAPWOOD OF PRUNED APRICOT TREES WITHIN AND OUTSIDE A SHELTER

Colonies isolated	Mean No. of colonies isolated per stem after:						
	3 days*	6 days	9 days	12 days	15 days	18 days	30 days
	Trees within shelter						
Bacteria	51·6	48·4	20·2	39·6	12·6	34·9	16·8
Cladosporium herbarum	25·6	152·1	196·1	211·3	272·8	164·5	209·1
Aureobasidium pullulans	3·9	0·6	4·8	5·4	2·9	20·8	0·2
Alternaria spp.	0·3	0·0	0·0	0·0	0·4	0·0	0·0
Penicillium spp.	0·0	5·2	2·0	0·4	1·3	0·0	0·4
Fusarium spp.	0·0	0·1	0·0	0·0	0·0	0·0	0·0
Other fungi	0·1	0·8	0·0	0·5	0·0	1·8	0·6
	Trees outside shelter						
Bacteria	97·0	249·9	219·8	287·4	148·8	203·9	213·4
Cladosporium herbarum	3·0	0·8	2·2	0·1	4·3	0·0	31·2
Aureobasidium pullulans	12·0	1·1	11·4	10·0	21·9	17·8	49·3
Alternaria spp.	0·2	0·1	0·0	0·1	0·0	0·0	0·0
Penicillium spp.	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Fusarium spp.	0·0	0·1	0·0	0·0	0·0	0·0	0·2
Other fungi	0·5	0·0	1·0	0·1	0·0	0·0	1·3

*Days after pruning.

The total number of colonies isolated increased rapidly up to 6 days after pruning with maximum numbers being recorded in each treatment at 15 days after pruning. The numbers showed a decline at 18 days, but had increased again at 30 days (Fig. 2), mainly due to increases in the fungal populations (Table 2). No differences were apparent between the treatments.

A total of 473 isolates (151 bacteria and 322 fungi) were tested for antagonistic activity towards *E. armeniacae*. Two bacterial isolates and 33 fungal isolates showed some activity; of the latter, 29 were species of *Fusarium*.

(*c*) *Colonization of Wounded Sapwood in the Presence or Absence of Benomyl*

The colonization of apricot sapwood following winter pruning was studied further in 1972. The effect of benomyl on this process was also investigated.

Selected leaders and laterals on four trees were pruned above a dormant bud on 30 June 1972. Immediately after pruning, two of the trees were sprayed to run-off with benomyl (50% a.i.; 0·507 g/l). Five stems from each tree were removed at

6, 12, and 18 days after pruning. The original pruned surfaces were first pressed gently against 5% Czapek–Dox agar in plates. The surfaces were then flamed and isolations from within the wood made as previously described.

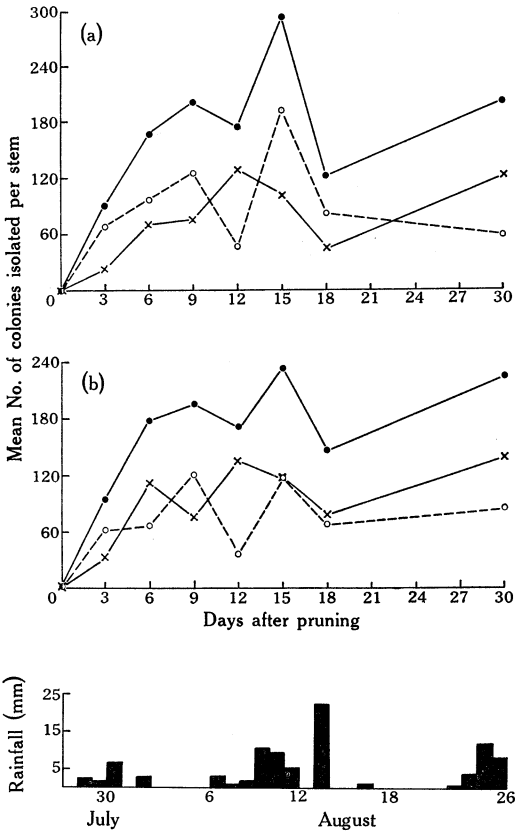


Fig. 2.—Colonization of sapwood of pruned apricot trees in the absence (*a*) or presence (*b*) of copper oxychloride. ● Total colonies. ○ Bacterial colonies. × Fungal colonies.

(i) *Surface Microflora*

Surface imprints of stems from unsprayed trees yielded bacteria and *Aureobasidium* colonies 6 days after pruning; these increased in number at 12 and 18 days. *Cladosporium herbarum* was recorded 12 days after pruning and was more abundant at 18 days.

Imprints from benomyl-sprayed trees yielded mainly bacteria, and fungi were virtually absent in comparison with those from the unsprayed trees (Figs. 4 and 5).

(ii) *Subsurface Microflora*

In both treatments very few colonies were isolated 6 days after pruning, but a large number was obtained at 12 days followed by a sharp decline at 18 days.

Bacteria were the predominant colonizers; few fungi were isolated and these were more abundant in stems from unsprayed trees (Fig. 3).

TABLE 2

BACTERIAL AND FUNGAL COLONIES ISOLATED FROM SAPWOOD OF PRUNED APRICOT TREES IN THE PRESENCE OR ABSENCE OF COPPER OXYCHLORIDE

Colonies isolated	Mean No. of colonies isolated per stem after:						
	3 days*	6 days	9 days	12 days	15 days	18 days	30 days
			Untreated				
Bacteria	66·1	96·5	125·3	44·3	192·9	81·9	58·0
Cladosporium herbarum	2·4	39·3	39·7	33·7	2·3	4·7	25·0
Aureobasidium pullulans	4·2	24·7	15·9	96·0	65·9	21·8	115·3
Alternaria spp.	0·2	0·0	0·1	0·3	1·3	1·3	0·1
Penicillium spp.	0·0	1·2	0·1	0·0	0·1	0·0	0·1
Fusarium spp.	0·4	0·4	0·1	0·0	0·0	0·2	0·1
Phoma spp.	0·1	0·3	0·2	0·4	0·0	1·8	0·0
Other fungi	0·0	4·1	0·0	0·0	0·0	0·0	0·0
			Treated with copper oxychloride				
Bacteria	72·2	66·3	120·3	39·2	117·2	68·2	85·9
Cladosporium herbarum	0·5	7·2	30·7	14·1	1·3	7·0	14·0
Aueobasidium pullulans	12·6	90·8	24·9	91·5	97·1	59·2	123·8
Alternaria spp.	0·1	0·5	0·7	0·5	0·7	0·5	0·5
Penicillium spp.	0·0	0·0	0·0	0·0	0·1	0·0	0·0
Fusarium spp.	0·0	0·1	0·7	0·0	0·1	0·4	0·6
Phoma spp.	0·3	0·3	0·5	0·1	0·5	0·0	0·2
Other fungi	0·0	0·8	0·0	0·0	0·1	0·0	0·0

*Days after pruning.

Two additional stems were removed from each treatment 12 and 18 days after pruning, and the wood particles bored from these were streaked on the surface of Czapek–Dox plates. The results (Table 3) confirm that benomyl caused a reduction in the population of bacteria obtained after 12 days and had strongly suppressed the fungal flora, especially *Aureobasidium pullulans.*

IV. DISCUSSION

Any analysis of a microflora, whether in soil, on leaf surfaces, or in sapwood, is limited by the methods used. Cultural methods inadvertently discriminate between organisms with different nutrient requirements and no one medium is entirely satisfactory for the isolation and study of an entire microflora (Dickinson 1971). Colonies arising in culture may originate from individual propagules or from a sporulating colony and it is likely that propagules from the latter may be redistributed by shaking and so yield a high count in the isolation plates.

Although there were technical limitations in the present study it has been possible to detect some treatment effects which clearly influence the pattern of colonization of the sapwood exposed by pruning apricot trees. On sheltered trees

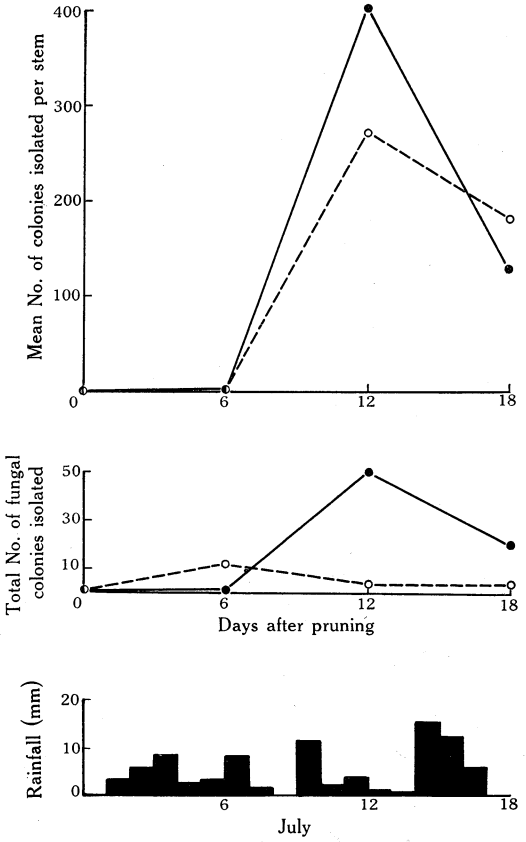
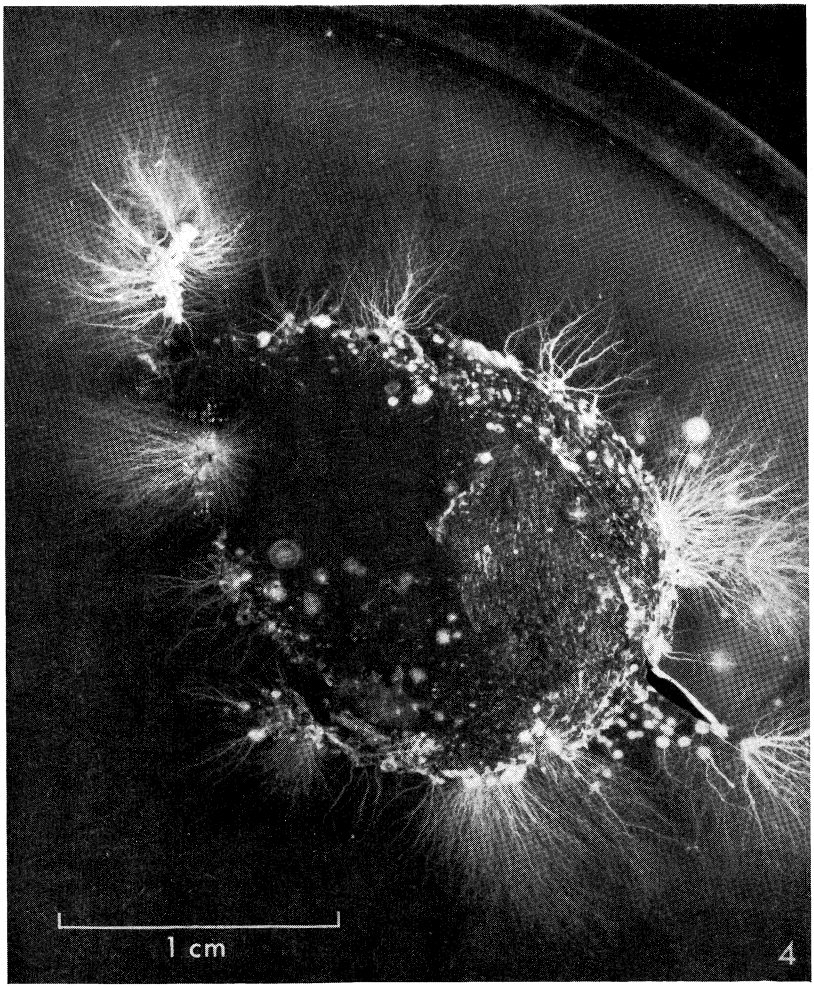


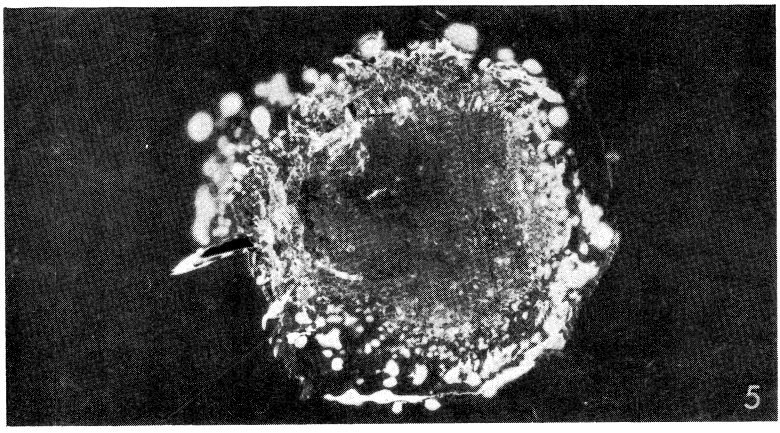
Fig. 3.—Colonization of sapwood of pruned apricot trees in the presence (○) or absence (●) of benomyl.

TABLE 3

NUMBER OF COLONIES OBTAINED FROM WOOD PARTICLES STREAKED ON AN AGAR PLATE

Treatment	Days after pruning	Total	Mean No. of colonies isolated per stem			
			Bacteria	Fungi	*Aureobasidium pullulans*	*Cladosporium herbarum*
Control	12	143	135	8	8	0
	18	151	86	65	57	6
Benomyl	12	66	65	1	1	0
	18	75	71	4	3	0





Figs. 4 and 5.—Imprint from pruned surfaces of unsprayed tree (Fig. 4) and of tree sprayed with benomyl (Fig. 5).

colonization was less rapid, and bacterial populations were significantly ($P = 0{\cdot}05$) smaller than on trees outside the shelter. This difference is attributed to the twofold effect of rainfall: first in conveying organisms to sapwood surfaces exposed on the pruned stems and second in providing the high moisture requirements for establishment there, of some species of the microflora.

The pattern of colonization on unsheltered trees resembled that found by Shigo (1967) in his study of succession of organisms on branch wounds of red maple; bacteria were the primary colonizers and were followed by fungi. The most interesting parallel, however, may be drawn with the data of Carter and Moller (1970) from their studies of the rate of decline in susceptibility of apricot sapwood to *E. armeniacae*. It is now apparent that susceptibility to this pathogen and increase in the population of saprophytic microorganisms in the immediate vicinity of the infection courts of *E. armeniacae* have a close inverse relationship. Although it is not yet possible to claim causality in this relationship the data presented here certainly provide strong support for the hypothesis advanced by these authors to explain the consistent rate of decline in susceptibility to *E. armeniacae* of winter-pruned apricot trees.

An interesting contrast has also been demonstrated between the effects on sapwood microflora of the two fungicides which are currently recommended for routine use in apricot culture in South Australia. Copper-containing fungicides have been used for many years and Carter (1971) advanced some circumstantial evidence in support of the belief that this practice may have favoured the chances for infection of trees by *E. armeniacae* at each annual pruning. In the present study, and in another experiment not reported here (Carter and Price, unpublished data), local application of copper oxychloride to individual wounds failed to yield a detectable effect either on the pattern of early colonization of wounded sapwood or on the proportion of pruned stems which can be infected by ascospores of *E. armeniacae* applied at intervals of up to 12 days after pruning. In normal commercial practice, however, trees are often sprayed with copper-containing fungicides. Furtado (1969) found that the pathogenic strain of *Colletotrichum coffeanum* was more abundant on coffee branches sprayed with a copper-containing fungicide than on those that were unsprayed, and Gibbs (1972) found that copper sprays applied early could increase sporulation of *Phoma* sp. by 49–121%. The effect of spraying trees with copper-containing fungicides thus needs further investigation.

With benomyl, however, a different situation obtains because of its wide spectrum of fungicidal activity and its lesser effect on the bacterial components of the microflora. Sapwood from stems harvested from sprayed trees yielded very few fungal colonies but the total bacterial population was relatively unaffected. It is not at present known whether this fungicide has any effect on infection by *E. armeniacae* ascospores which arrive at the infection court at periods later than 1 day after pruning.

The influence of the saprophytic tree microflora on infection by *E. armeniacae* is uncertain. The rapid colonization of sapwood following pruning could lead to fewer infection courts being available for the pathogen. About 7% of the isolates tested showed some *in vitro* antagonism to *E. armeniacae* and these were mostly

species of *Fusarium*. The *in vitro* test was designed to detect inhibition of fungal growth and the effect of these organisms on ascospore germination was not investigated. It is possible that bacteria, which were abundantly present, could inhibit spore germination. Blakeman and Fraser (1971) showed that the germination of conidia of *Botrytis cinerea* Pers. ex Fr. on the surfaces of chrysanthemum leaves was inhibited by the presence of bacteria. A strain of *Aureobasidium pullulans* also reduced the number and length of germ tubes of *Alternaria zinniae* Pape (Van den Heuvel 1969). However, *A. pullulans* did not show any antagonism to growth of *E. armeniacae* and was rarely found on trees sprayed with benomyl.

Further work on the effects of specific components of the microflora colonizing apricot pruning wounds is necessary as this may lead to more effective control of the pathogen by biological methods.

V. Acknowledgments

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