BACTERIAL SPOT OF STONE FRUIT IN QUEENSLAND

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Abstract

Bacterial spot, *Xanthomonas pruni*, is a serious disease of stone fruit in the Granite Belt district of south-eastern Queensland. The Queensland isolates of *X. pruni* correspond to those described from overseas. Isolations from summer cankers on current seasons' plum and peach laterals showed that the organism survives the winter in a small number of cankers to provide one source of spring inoculum. The inability of the organism to survive in all cankers appears to be associated with the sealing-off of diseased tissue by periderm.

Results from internodal inoculations of plum and peach laterals during late summer and autumn showed that the susceptibility of the tissue to invasion by *X. pruni* increases with the commencement of leaf fall. Infection at that time could provide another source of spring inoculum.

It was concluded from the work carried out that, on the Granite Belt, summer cankers provide one source of spring inoculum and any fresh infections taking place after the commencement of leaf fall, e.g. via exposed leaf scars or natural openings, could result in another source of inoculum for the spring.

I. INTRODUCTION

The stone fruit industry in Queensland is centred in the Granite Belt district in the south-east of the State. Although only 5° south of the Tropic of Capricorn the district has an elevation of 2500–3500 feet above sea level which provides the necessary winter chilling requirements for stone fruit production. The annual rainfall of 30 in., 59% of which falls in the period from September to February, is also satisfactory (Ward 1952*a*, 1952*b*). Heavy dews are frequent during autumn.

Bacterial spot [*Xanthomonas pruni* (Erw. Smith, 1903) Dowson, 1939] was first recorded in Queensland on plum in 1930 and on peach in 1965. As a result of field trials and accumulated evidence, Morwood (1947) recommended three sprays with copper sulphate from bud movement to full blossom for the control of this disease. Despite these measures the disease is still of considerable importance in the district and fruit losses in certain years are high. Badly affected trees are characterized by a gradual loss of leaders from season to season until the tree becomes economically unproductive.

Bacterial spot has only been found in Queensland and New South Wales. In the latter State it was recorded on plum and peach in 1929 and 1960 respectively. Hutton (personal communication, 1971) considered that the strain on peach could be different from that on plum.

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Following a severe outbreak of bacterial spot in the spring of 1968 work was commenced to study the aetiology of the disease and to characterize the strain or strains of *X. pruni* involved. Emphasis was placed on determining the means of overwintering and the source of spring inoculum.

II. Materials and Methods

(*a*) *Field Observations*

The annual growth cycle of plum and peach trees is as follows: a dormant period from April until August, blossoming from late August until September, shoot growth from September until March, harvesting December until February, leaf fall from March until May.

Observations of the symptoms and development of the disease reported in this paper were made in badly affected plum cv. Doris and peach cv. Elberta orchards from October 1968 until October 1970. General surveys were also conducted in stone fruit orchards throughout the Granite Belt.

(*b*) *Isolation and Pathogenicity of the Causal Organism*

Isolations were made from leaf spots, cankers on laterals, the nodal region of laterals, fruit spots, and plum floral parts. The medium used for isolations was sucrose peptone agar (SPA) (Hayward 1960).

Pathogenicity tests were performed either by spraying a suspension of the organism in sterile distilled water onto leaves using a hand atomizer or by pricking a drop of the suspension into current season's laterals and green plum fruit. The inoculated area was enclosed in clear plastic sheeting or, in the case of pricked fruit, kept in a moist chamber for approximately 24 hr.

(*c*) *Identification of the Organism*

Three pathogenic isolates from plum and two from peach were characterized using methods previously described by Moffett and Colwell (1968) except for starch hydrolysis and hydrogen sulphide production from cystine. These two tests were performed using the methods described by Skerman (1967). Sucrose peptone basal medium was employed in all determinations.

(*d*) *Survival of the Pathogen*

Laterals with suspected bacterial spot summer cankers (Thornberry and Anderson 1933) were collected at random from plum and peach trees during the summer and winter months. Collections from plum extended from February 1969 to October 1970, and those from peach from February 1969 until July 1969. The collections were made at least twice during each month. The nodes of these laterals were examined also for any discoloration. Isolations were made from all cankers and also from nodal tissue suspected of being diseased.

(*e*) *Histological Examination of Summer Cankers*

Laterals of plum and peach with current season's summer cankers were collected each month from November 1969 until leaf fall in 1970. A section of the lateral which included the margin of a summer canker and the adjacent healthy tissue was embedded in paraffin and sectioned using a sledge microtome. The plant tissue was fixed in formalin–acetic acid–alcohol, dehydrated using t-butanol (Johansen 1940), and stained with thionin and orange G (Stoughton 1930). Serial sections were examined.

(*f*) *Determination of Susceptibility of Plum and Peach Tissue to Infection during late Summer and Autumn*

A trial was carried out to determine whether the current season's tissue of plum and peach was susceptible to *X. pruni* during late summer and autumn. Plum cv. Wilson (moderately susceptible) and peach cv. Golden Queen (susceptible) growing at the Granite Belt Horticultural Research Station were used.

Inoculations were made at monthly intervals from February until June when leaf fall was complete. One drop of a suspension of a 48-hr-old culture in sterile distilled water, adjusted turbidimetrically to give a concentration of 10^8 bacteria per millilitre, was inoculated into the internodes of current season's growth on 3-yr-old trees with a hypodermic syringe. This was carried out by splitting the bark slightly and placing the drop of suspension on the broken surface beneath the bark. Inoculated laterals were then enclosed in clear plastic sheeting overnight to prevent desiccation. Sufficient inoculations were made to ensure that one inoculated area could be sampled at fortnightly intervals until 10 October. The tests were repeated on four replicate trees. Isolations were made from each inoculated area and the degree of penetration and spread, determined by discoloration beyond the point of inoculation, was noted.

III. Results

(*a*) *Field Observations*

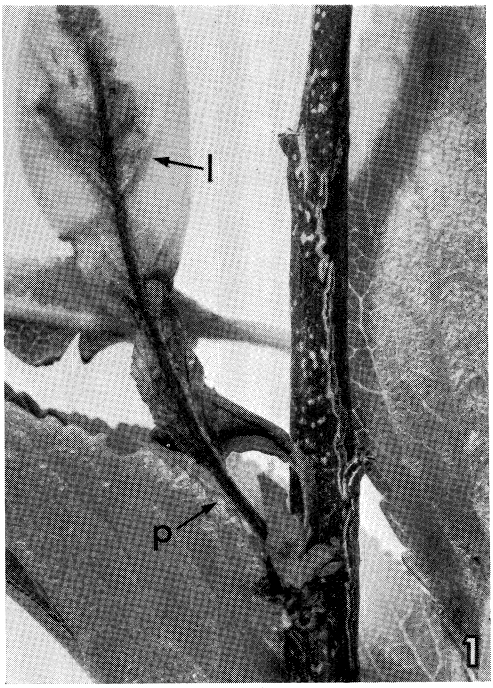
The stem cankers and fruit symptoms corresponded to those described and well documented by overseas workers (Rolfs 1915; Dunegan 1932; Hopperstead and Manns 1945; Reid 1945). Leaf spots appeared in early October as greasy or water-soaked angular spots which dried to a light tan colour, becoming dark brown to black as they aged. Very soon the diseased tissue separated from the surrounding healthy tissue and eventually dropped out to form a shot-hole symptom. Adjacent spots often coalesced and, where infection was heavy, the affected area of the leaf became chlorotic. Severe leaf spotting resulted in premature leaf fall. While the discrete leaf spot symptoms were similar to those described above and elsewhere, the first observable foliage symptom on the plum trees at the beginning of October 1969 appeared rather different in that many of the emerging leaf whorls were stunted and blighted. Areas of the leaf lamina adjacent to the midrib of the expanding new leaves together with the petiole were often darkly discoloured. In some instances the leaves failed to unfurl (Figs. 1 and 2). The more typical leaf spots appeared soon afterwards.

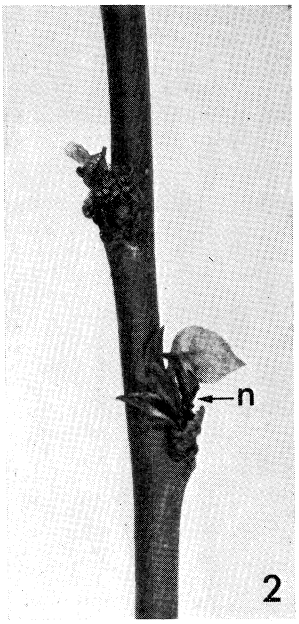
The summer cankers (Thornberry and Anderson 1933) appeared after the leaf symptoms were well developed. They were first obvious as elongated water-soaked light tan lesions which extended lengthwise along the lateral. They became depressed, ruptured, and darkened in colour. With age the margin of the canker became raised above the adjacent healthy tissue and the diseased area took on a gnarled appearance. Infection of the succulent young lateral sometimes occurred at adjacent points, which resulted in a girdling effect and premature death of the young shoot.

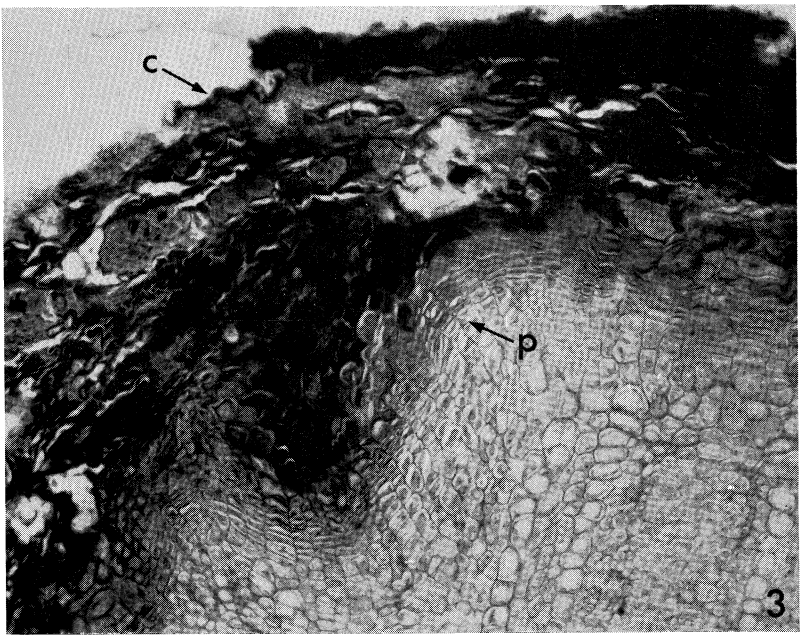
Fruit lesions on plum commenced as water-soaked circular spots which rapidly enlarged and cracked as the fruit developed. The centre of the spots darkened to a purplish black colour. On peach they were at first pinpoint water-soaked spots with adjacent spots coalescing. Individual spots became tan with a water-soaked margin. As the fruit enlarged cracks appeared and a gummy exudate was produced.

Fresh leaf infections continued throughout the whole growing season following periods of moist, dull weather. However, new infections of the plum laterals were rarely observed after January and fruit appeared to become resistent 4–6 weeks before harvest. Peach cankers were sometimes difficult to find until the end of January.

In a survey carried out on the Granite Belt during the spring of 1969, it was found that bacterial spot was widespread throughout the district, in which the soil type in which stone fruit are grown ranges from a coarse sand to a fine sandy loam.







Figs. 1 and 2.—Young leaves of plum cv. Doris blighted and stunted by *X. pruni*. *l*, Discoloured area of leaf lamina; *p*, petiole discoloured; *n*, leaves discoloured, stunted; such leaves failed to expand.

Fig. 3.—Transverse section through a plum lateral cv. Doris; periderm enclosing a canker produced by *X. pruni*. *c*, Canker area; *p*, periderm. × 35.

Matthee and Daines (1968) found that the peach trees cultivated on sandy soils were more susceptible to bacterial spot than those grown in a heavier soil. Although a number of cultivars of plum and peach were affected, it was not present in all orchards growing susceptible types. No cultivars of English origin showed the disease. The Japanese cultivars examined, in order of decreasing susceptibility, were Doris, October Purple, Santa Rosa, Shiro, Mariposa, and Burbank, with Wilson being only moderately susceptible. The order of susceptibility of peach cultivars was Elberta, Starking Delicious, Southland, J. H. Hale, Halehaven, Golden Queen, Pullar's Cling, and Wiggins. Cultivars of apricot, nectarine, and cherry were also found affected by the disease.

(*b*) *Isolation and Pathogenicity*

A bacterium forming yellow mucoid colonies on SPA was consistently isolated in large numbers from leaf spots until leaf fall was complete, and from cankers, nodes, floral parts, and fruit spots. The pathogenicity of a number of plum and peach isolates was confirmed and these were used in subsequent experiments. Cross-inoculations of plum and peach isolates onto plum and peach leaves in the field and inoculation of both isolates onto plum fruit in the laboratory indicated that only one strain was involved.

(*c*) *Identification of the Organism*

The bacterium was Gram-negative, rod-shaped, 2·4–1·8 by 0·9–0·6 μm in size, motile, with a single polar flagellum. On SPA, colonies were circular, smooth, raised, margin entire, and mucoid with a yellow water-insoluble pigment. In sucrose peptone broth turbidity after 96 hr was moderate. The optimum temperature of growth was in the range of 23–26°C. The organism could tolerate 3% but not 5% NaCl. All isolates were sensitive to tetracycline, chlortetracycline, oxytetracycline, erythromycin, polymyxin B, dihydrostreptomycin, kanamycin, and novobiocin, but resistent to penicillin. Glucose, fructose, lactose, L-arabinose, cellobiose, raffinose, mannose, galactose, sucrose, trehalose, melibiose, and glycerol were oxidatively metabolized by all isolates and xylose by one peach isolate. Rhamnose, maltose, salicin, ribose, mannitol, sorbitol, erythritol, dulcitol, inositol, dextrin, inulin, methyl gluconate, and 5% ethanol were not metabolized. Litmus milk was peptonized. All isolates gave a negative test for tyrosinase, arginine dihydrolase, and oxidase but a positive one for catalase and phosphatase. Casein and aesculin were hydrolysed, ammonia was produced from peptone, and hydrogen sulphide from cystine. Nitrate was not reduced and starch was not hydrolysed.

(*d*) *Survival of the Organism*

A summary of the results of isolations from summer cankers and nodes on laterals collected during the period from February 1969 until October 1970 is given in Tables 1 and 2. These results show that there was a reduction in the number of cankers containing viable bacteria during the period from February until June. However, the organism remained viable throughout the winter months in a few of these cankers. From July the organism was isolated from a greater percentage of

cankers sampled. Also there was an obvious renewal of activity from the margin of the cankers, with observable extension into the cortical tissue beyond the area of the

TABLE 1

OCCURRENCE OF *X. PRUNI* IN SUMMER CANKERS AND NODES ON LATERALS OF DORIS PLUM SAMPLED AT INTERVALS FROM FEBRUARY 1969 UNTIL OCTOBER 1970

Month of sampling	Cankers on current season's laterals		Cankers on 2nd-yr wood		Nodes on current season's laterals		Spurs on 2nd-yr wood	
	No. examined	No. with *X. pruni*	No. examined	No. with *X. pruni*	No. examined	No. with *X. pruni*	No. examined	No. with *X. pruni*
1969								
Feb.	5	3	—	—	—	—	—	—
Mar.	17	4	2	0	—	—	—	—
Apr.	52	20	6	0	—	—	—	—
May	42	4	—	—	21	3	2	0
June	27	2	—	—	29	4	9	4
July	30	9	—	—	11	5	13	4
Aug.	38	17	—	—	41	7	14	6
Sept.	51	15	1	0	10	5	2	2
Oct.	6	3	—	—	2	1	—	—
1970								
Feb.	48	14	—	—	16	3	2	0
Mar.	40	14	3	1	1	0	1	1
Apr.	43	5	2	0	16	8	3	1
May	15	1	—	—	14	3	—	—
June	10	2	—	—	16	2	—	—
July	13	7	2	1	42	5	6	0
Aug.	10	4	3	0	35	12	—	—
Sept.	7	4	—	—	30	18	—	—
Oct.	6	5	—	—	6	6	—	—

original canker to form the spring cankers (Thornberry and Anderson 1933). Where an area of the canker was still active it was usually moist and light tan with a diffused

TABLE 2

OCCURRENCE OF *X. PRUNI* IN SUMMER CANKERS AND NODES ON LATERALS OF ELBERTA PEACH SAMPLED AT INTERVALS FROM FEBRUARY 1969 TO JULY 1969

Month of sampling	Suspected summer cankers		Nodes	
	No. examined	No. with *X. pruni*	No. examined	No. with *X. pruni*
February	4	0	—	—
March	13	1	—	—
April	60	2	7	0
May	43	0	22	0
June	41	1	39	0
July	59	5	25	2

margin. In spring, cracks in the bark were observed over these freshly invaded areas. In contrast, inactive cankers were dark to reddish tan in colour, dry, with margins

distinctly demarcated from adjacent healthy tissue. Only a small percentage of cankers sampled on 2-yr-old wood contained viable bacteria.

The organism was obtained from nodes and spurs on 2-yr-old wood. In many instances the discoloration at the node did not appear to be a continuation of a canker. In fact there were instances where infection of this area seemed to occur as a result of infection in the leaf scar. Sometimes only one side of the node appeared to be affected whereas in the extreme case the whole area was water-soaked and discoloured. In some instances there was external indication of infection.

(*e*) *Histological Examination of Summer Cankers*

Periderm was observed to be forming between the diseased area and adjacent healthy tissue as early as December. In the following months this layer became well developed and conspicuous (Fig. 3). Not all the diseased tissue was completely sealed off by this barrier because areas of invaded cortical tissue were sometimes observed outside the main canker.

TABLE 3

RESULTS OF INOCULATING *X. PRUNI* BENEATH THE BARK OF WILSON PLUM AND GOLDEN QUEEN PEACH FROM FEBRUARY 1969 UNTIL JUNE 1969

Date of inoculation	No. of sites*	Positive isolations (%)	
		Plum	Peach
26.ii.69	16	29·7	15·6
13.iii.69	15	13·6	20·0
27.iii.69	14	33·9	57·1
10.iv.69	13	17·3	65·5
24.iv.69	12	37·5	41·7
9.v.69	11	63·6	70·5
5.vi.69	9	97·2	—

* Isolations made at fortnightly intervals from each inoculation site.

(*f*) *Determination of Susceptibility of Plum and Peach Tissue to Infection during late Summer and Autumn*

The extent of invasion by the organism of the tissue from the point of inoculation varied. With some exceptions the discoloured area was contained between the two consecutive buds. Table 3 gives a summary of the results of isolations from inoculations made between February and June 1969. Few organisms survived in lesions as a result of the February, March, and April inoculations. This was reflected by the fact, that in most instances, less than 10 colonies grew on the isolation plates. The organism was isolated from a greater percentage and in much greater numbers from the later inoculations. In fact, from August on, the appearance of affected tissue produced as a result of inoculations at the beginning of May and later resembled the active margin of natural cankers at the same period.

IV. Discussion

The characteristics of the Queensland isolates were in agreement with the description of *X. pruni* by Hayward and Waterston (1965). There was no significant difference between any of the isolates examined. The fact that *X. pruni* was not recorded on peach in New South Wales and in Queensland until 1960 and 1965 respectively could have been due to the appearance of a new strain of the organism. Although Hutton considered that at least two strains were present in New South Wales, one specific to plum and the other specific to peach, limited cross-inoculation tests carried out in this study indicated that this may not be the case in Queensland. More work will have to be carried out to clarify this point.

Results from these investigations show that the organism can survive the winter months in summer cankers on plum and peach, which provide a source of inoculum for the following spring. This is in agreement with evidence elsewhere (Adams 1926, 1929; Dunegan 1932; Foster and Peterson 1954). Wilson (1939) discussed the phenomenon of active and inactive cankers in bacterial canker of stone fruits. Similarly, in bacterial spot certain cankers remain active and from these cankers the organism advances into the adjacent healthy tissue in late winter. During wet periods or heavy dews in the spring, bacteria from this freshly affected tissue (spring cankers) ooze through openings in the bark to provide inoculum for primary spring infection. The active margin of such cankers corresponds with those areas not cut off from healthy tissue by a periderm. This periderm seems to form a protective barrier isolating the diseased tissue, with the eventual death of most of the contained bacteria. Thornberry and Anderson (1933) also noted this sealing-off of diseased tissue from healthy tissue.

Infection during autumn via the leaf scar was discussed for peach by Feliciano and Daines (1970) and mentioned in plum by Matthee (1968). During the examination of the nodal region of plum and peach laterals it was noted that there were instances where infection appeared to have occurred by way of the leaf scar and it is considered that this type of infection may be of some importance in the Granite Belt. It is considered that the leaf blight symptom of plum observed in the 1969 spring may have been principally a result of heavy autumn leaf scar infection.

The tissue of both plum and peach becomes more susceptible to *X. pruni* about the commencement of leaf fall, and, as the dormant period progresses, spread of the organism from existing cankers or other sources of infection is accelerated. Provided the organism can gain entry into plum and peach tissue following the commencement of leaf fall, then invasion of the cortex and other tissue can occur.

This study has shown that the etiology of bacterial spot on stone fruit in Australia is comparable to the findings reported by overseas workers. It established the importance of summer cankers in the overwintering of *X. pruni* and the presence of viable bacteria in leaf spots until the completion of leaf fall; it also showed that any infection taking place from the commencement of leaf fall either via leaf scars or natural openings can result in a further increase in inoculum concentration in the tree at the end of dormancy. For these reasons bacterial spot is difficult to control. Any control measure recommended should aim at protecting the emerging vegetative shoots from spring infection, preventing the formation of summer cankers, and protecting of exposed leaf scars and natural openings during the autumn.

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