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A THESIS

PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF HORTICULTURAL SCIENCE

AT MASSEY UNIVERSITY

EVALUATION OF SEAWEED EXTRACT AND TWO RESISTANT PEACH VARIETIES FOR CONTROL OF PEACH LEAF CURL DISEASE *(TAPHRINA DEFORMANS)*

December 1989

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ACKNOWLEDGEMENTS

I am indebted to a number of people for the completion of this study. In particular I must thank my supervisor Dr Peter Long for his assistance throughout the project. Thanks also to the technical staff of the Plant Health Department, especially Mrs Lorraine Davis for assistance in the tree planting and Mr Hugh Neilson for help and advice with laboratory tests. The analysis of statistics would not have been possible without the patient help of Dr Hugo Valera-Alvarez, for which I am most grateful.

I am appreciative of the support from the industry: Mr Vern Harrison for the supply of the peach varieties tolerant to peach leaf curl; Mr Robert Porteous for the supply of the Seagro seaweed extract and to Growth Marketing Ltd. for the supply of Response and other seaweed extracts. Thanks also to Dr Cheah of the Levin Research Centre for the supply of the *Taphrina deformans* isolate.

I am grateful for the support from my colleagues in the Department of Agricultural and Horticultural Systems Management and for the use there of computing facilities.

Finally, I thank my husband, Brian Fisher, whose support, understanding and very persistent harassment enabled the completion of this thesis.

ABSTRACT

The effectiveness of seaweed extracts for the control of peach leaf curl (*Taphrina deformans)* on Red Diamond nectarines was compared with a copper fungicide and with two varieties of peach claimed resistant to peach leaf curl. Field and laboratory tests were undertaken.

The peach varieties Winiata Gold and Gordon's Glory were tolerant to peach leaf curl under conditions of severe disease pressure. They were more effective than seaweed extracts in the control of the fungus.

The seaweed extract, Seagro, did not control peach leaf curl disease on Red Diamond nectarines planted in a randomised complete block design in Palmerston North in 1987. No evidence of fungicidal properties was found in the second year of the study when there were eleven infection periods suitable for the leaf curl fungus. In this season both seaweed extract and copper oxychloride fungicide were ineffective. In laboratory experiments, the seaweed extracts Response, Ecklonia and Carpophyllum had no effect on *T. deformans* growth.

In vitro, *T. deformans* grew well on a media of potato dextrose agar and 1% peptone. Satisfactory growth was also obtained on whole potato extract but the ease of preparation of the commercial agar mix made it the best option for this study. *T. deformans* grew more slowly on malt agar and peptone and did not grow at all on Czapek media. Acidifying the PDA media to a pH of 3.5 did not significantly affect *T. deformans* growth.

Quick laboratory tests for comparison of fungal growth on different fungicide/seaweed extract amended PDA plates were compared. The measurement of light absorbance of cell suspensions with a spectrophotometer was quicker than counting cells using a haemacytometer. The petri dish zonal inhibition technique was useful for quantifying the effectiveness of some fungicides against *T. deformans.*

Streptomycin, Benlate, and Rovral had little or no effect on the growth of *Taphrina deformans in vitro* but Saprol inhibited growth.

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1.0 INTRODUCTION

Peach leaf curl disease has been a problem of peach growing since the early nineteenth century (Atkinson 1968) and occurs throughout New Zealand wherever peaches and nectarines are grown. It is common on peach and nectarine cultivars and has also been found on almonds. On the latter host however it is reportedly of no economic significance (Heyns, 1965).

On unsprayed trees leaf curl can become a serious disease. It may destroy the first leaves and much of the crop year after year, gradually weakening the tree until it dies or is invaded by other organisms (Atkinson 1968). However, for nearly a century leaf curl has been fairly easily controlled with fungicidal sprays such as copper-containing Bordeaux mixture and copper oxychloride.

In 1987 growers in Hawke's Bay New Zealand found that despite a regular spray programme leaf curl had become more of a problem each season. It was suggested that the cause of the problem was either fungicide resistance or the development of a strain of the leaf curl pathogen which causes infections later in the growing season. Tate *et al.* (1987) did not find any evidence of fungicide resistance or of an unusual strain of the leaf curl pathogen. They concluded that the infection period for Fantasia nectarines occurred later than was usual for stonefruit (at the end of August in 1987 and mid-September in 1986), compared with the traditional timing of spray application in July/early August. These were respectively four and six weeks too early. However, in 1989 a strain of *Taphrina deformans* was isolated by Cheah and Tate that had low sensitivity to copper fungicide in laboratory tests. In controlled greenhouse experiments this strain of *Taphrina deformans* caused leaf curl on copper sprayed nectarine trees. Further tests are underway to confirm that this represents true resistance to copper fungicides.

There is thus a need for an alternative to copper sprays for the control of peach leaf curl.

In addition to the problems encountered by New Zealand stonefruit growers research into an alternative to copper fungicides is relevant for two other reasons: (i) the need for organic growers to decrease the use of such fungicides if their products are to be certified as organically grown, and (ii) the potential problem of copper reaching toxic levels in orchard soils as a result of long term use.

(i) Organic growing

To obtain certification of organic produce organic growers in New Zealand follow the guidelines set by the Biological Producers Council (BPC). These state, with respect to pest and disease control, that the

routine use of any pesticide is not allowed. Plant protection treatments based on natural plant extracts and/or simple salts may be used. Prohibited pesticides include all organochlorine, organophosphate and carbamate insecticides, dithiocarbamate fungicides and other synthetic pesticides.

To prevent peach leaf curl organic growers may use copper fungicides approved by the BPC, provided they are not used 'routinely'. The only copper fungicide specifically approved in the 1988 BPC standards is cupric hydroxide but it is known that organic growers in New Zealand may also use copper oxychloride. For growers to retain organic certification of their crops their use of fungicides must decrease over a period.

(ii) Copper toxicity

Potential copper toxicity problems have been documented by Merry *et al.* (1983). They found the level of copper, lead and arsenic was up to 25-35 times higher in soils from orchards and former orchards in South Australia and Tasmania than in uncontaminated agricultural soils. The high affinity of copper for oxide minerals and organic matter resulted in the retention of copper in the surface 20-30 cm of soil with no evidence of leaching to greater depth, even in the acid, sandy soils of Tasmania.

However, they also found that the concentration of copper in pasture plants (ryegrass and white clover) growing on former orchard soils in Tasmania was generally unrelated to total soil concentration of copper. In South Australia other pasture plants (from former orchard soils) had concentrations much higher than that recommended for sheep grazing - up to 20 ppm. The warmer soil temperatures of South Australia account in some way for this as plants accumulate more copper at higher temperatures (Merry *et al.* 1983).

Pasture plants in both studies had no obvious symptoms of toxicity, even where copper levels were up to 50 ppm. This is significant given the large number of former orchards in Australia which support pastures, and the high number of existing orchards with high concentrations of copper in the soil which support good stands of grasses (usually with legumes) as ground cover.

Despite these Tasmanian and South Australian results of Merry *et al.* earlier work by Merry (in Merry *et al.* 1983) found a relationship between soil concentrations of lead and copper and those in pasture, crop, and vegetable plants grown in contaminated soils in field and glasshouse experimental plots. The lack of a simple correlation between plant and soil levels of copper in the latter experiment may be due to the effects of other toxic elements, fertilisers and different acidities of contaminated soils. Plant uptake of copper increases with increased acidity (Merry *et al.* 1983).

Merry 's glasshouse experiments indicated that the copper, lead and arsenic contents of vegetables grown on former orchard soils would not exceed established standards for human consumption (in Merry *et al.* 1983). He cited two potentially dangerous circumstances for humans: people growing, and largely consuming, their own vegetables on highly contaminated former orchard soils and children in such areas ingesting dirt. To the author's knowledge, no study has been done in New Zealand on the concentration of copper in orchard soils. However, in general, much of New Zealand's soils are copper deficient.

Two techniques which would be acceptable to organic growers for the control of leaf curl disease are the use of resistant varieties and the use of plant or seaweed based products. There has been interest in the use of seaweed extracts for the control of leaf curl of peaches by organic growers (Chase 1986) but there is limited research in this area. No detailed observations have been made on the relative resistance to leaf curl of locally grown stonefruit varieties, but it is known that most of the varieties grown for export are susceptible in some degree (Atkinson 1968).

This work was undertaken to evaluate the effectiveness of seaweed extracts for the control of peach leaf curl and to assess the resistance of some "leaf curl resistant" stonefruit. A review of literature on the fungus *Taphrina deformans* was made and its classification, biology and lifecycle, culture ***in vitro*** and control is documented in section 2. Laboratory experiments were used to develop a technique for the growth of *T. deformans* in culture and for a method to evaluate the effectiveness of products in the control or inhibition of the fungus. These are described in section 3. A field trial was set up to compare the effect of a seaweed extract with a copper fungicide for the control of peach leaf curl on Red Diamond nectarines. Two peach varieties, Gordon's Glory and Winiata Gold claimed to be resistant to leaf curl were assessed. A discussion of results is given in section 5.

2.0 LITERATURE REVIEW OF THE FUNGUS *TAPHRINA DEFORMANS*

In this section a review of literature on the peach leaf curl fungus is made. The classification of the fungus is given in section 2.1 followed by a description of the biology and lifecycle in section 2.2. The culture of *T. deformans in vitro* is documented in 2.3. Methods used to control peach leaf curl are described in 2.4 and include use of resistant varieties, fungicides and seaweed extracts.

2.1 CLASSIFICATION

The leaf curl fungus, *Taphrina deformans* has the following classification:

DIVISION Amastigomycota

-produce a well developed mycelium consisting of septate or aseptate hyphae.

SUBDIVISION Ascomycotina

-have a septate mycelium and produce ascospores in asci.

CLASS Ascomycetes (the sac fungi)

-their distinguishing feature is the ascus, a sac-like cell containing usually eight ascospores (Alexopoulos and Mims 1979). This sexual reproduction stage is produced on or in infected leaves, fruits or stems at the end of the growing season or when the food supply is diminishing.

-their other reproductive phase is the conidial or asexual stage (imperfect) in which most infections are caused.

SUBCLASS Hemiascomycetes

-have naked asci, ie. there is no protective fruiting body. Asci are formed directly from zygotes or single cells and have no ascogenous hyphae or ascocarps.

-Other orders in this subclass are the Protomycetales, which have asci in a reputedly common spore sac, and the Endomycetales which have asci formed into an ascus or a diploid ascophore (Martin 1940).

ORDER Taphrinales

-asci arise from binucleate ascogenous cells (Alexopoulos and Mims 1979). All are parasitic on vascular plants and causemalformations of the tissues they attack. They ressemble the yeasts in that the ascospores multiply by budding, as do yeast cells. They differ in that they produce a definite true mycelium (Alexopoulos and Mims 1979) in host tissue.

The present classification of the Taphrinales follows the decision by Mix (1949) to include all species under the one genus, *Taphrina*. However in the past this group has been divided in various ways and included the generic names *Ascomyces, Ascosporium, Exoascus, Magnusiella* and *Sarcorhophalum.*

T. deformans is the best known species of the Taphrinales. Other economically important species are *T. pruni*, the cause of plum pocket; *T. cerasi*, the cause of witches broom of cherries; *T. populina*, the cause

of puckering of poplar leaves and *T. coerulescens*, the cause of curling and puckering of oak leaves.

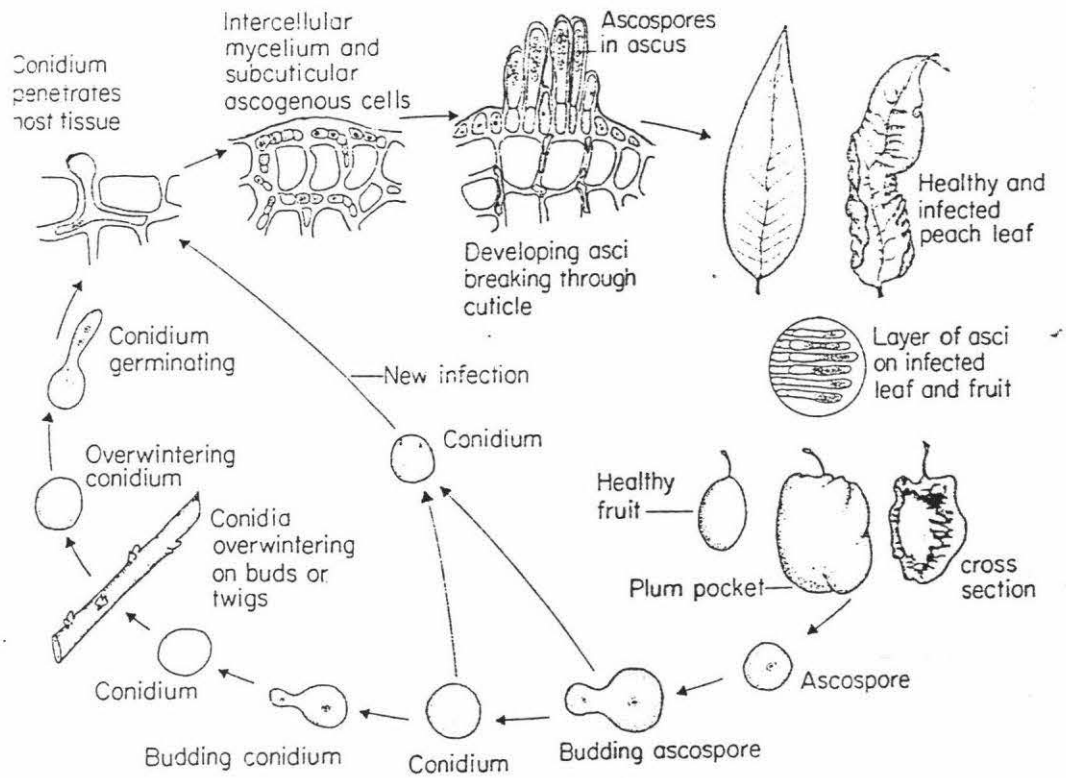
2.1.1 Summary

The distinguishing classification features of *T. deformans* are the lack of a protective fruiting body (ie asci are naked) and the multiplication of ascospores by budding, which also gives them a strong ressemblance to yeasts.

2.2 BIOLOGY AND LIFECYCLE

T. deformans is dimorphic. In its parasitic stage, within the host plant, it forms mycelium and naked asci and in its epiphytic stage yeast-like cells are budded. Figure A (Agrios 1978) shows the disease cycle of diseases caused by *Taphrina* species.

Figure A



Disease cycle of diseases caused by *Taphrina* sp.

The mycelial cells of *T. deformans* contain two nuclei and may develop into an ascus containing eight uninucleate ascospores. The ascospores multiply by budding inside or outside the ascus producing small, round or ovoid conidia (blastospores). These are uninucleate and haploid. They may bud again to produce more thin or thick-walled blastospores or may germinate to produce mycelium. Upon

germination, the conidial nucleus divides and two nuclei move into the germ tube. As the mycelium grows, both nuclei divide concurrently, producing the binucleate cells of the mycelium. Mycelial cells near the plant surface separate from each other and produce the asci (Agrios 1978).

There is still doubt as to how this fungus survives from one season to the next. For many years it was believed that perennial mycelium survived through winter in shoots and infected new leaves in spring. This idea was rejected for a number of reasons. Mycelium of *T. deformans* has not been found in twigs older than the current season's growth nor has it been traced from a diseased twig out into the new foliage shoot arising from it (Mix 1935). Secondary spores grown in culture can survive through a winter on peach shoots and cause spring infections, but attempts to isolate naturally deposited spores from dormant trees have been largely negative (Atkinson 1968).

There is no evidence that the fungus overwinters in leaves severely infected with leaf curl (Booth 1981). After heavy infestation defoliation of the host occurs and the leaves, now brown, continue to produce asci and blastospores but no (or very little) infection occurs on the second leaf crop (Booth 1981).

It is now thought that the fungus overwinters as ascospores or thick-walled blastospores which shelter in the bud scales of the host (Smith *et al.* 1988). This hypothesis is supported by the fact that a single fungicide spray at bud break can give almost complete control of leaf curl. In spring these overwintering spores are rain-splashed onto exposed, developing leaves and infection of the peach or nectarine occurs. Most primary infections occur on the lower leaf surfaces because as the young peach leaves emerge in spring only the undersides are exposed (Atkinson 1968).

The growth of the fungus after the initial penetration of the host has been well documented (for example in Mix 1935, Kramer 1973, Syrop 1975). An intercellular mycelium grows through the mesophyll and forms a thin sheet between the palisade layer and upper epidermis (Booth 1981).

T. deformans is a biotrophic parasite and does not kill the host cells in the first stages of infection but instead stimulates them into immediate cell division (hyperplasia) followed by cell enlargement (hypertrophy) and cell differentiation. The hyperplasia and hypertrophy caused by the fungal penetration mean that affected areas grow at a faster rate than the normal cells and this causes the typical leaf curling and distortion, as illustrated in Plate 1.

PLATE 1.

Peach leaves infected with *Taphrina deformans* showing typical symptoms

(Photo: courtesy of Dr P.G.Long)

All starch contents of an infected leaf are used by the fungus, and with gradual degeneration of chloroplasts a dark red colour appears in place of normal green as the cells develop anthocyanins (Booth 1981). As growth of the fungus proceeds midribs become swollen and curved inwards at their ends, while leaf blades become swollen and folded with edges curled downwards. *T. deformans* has a close nutritional relationship with the host along the complex cell wall/plasma membrane, because it is only through this barrier that it can obtain food for survival and propagation (Bassi *et al.* 1984).

Studies have shown that the peach leaf curl fungus releases both auxins (Crady *et al.* 1959; Trione 1964; Somner 1961) and cytokinins (Johnston *et al.* 1974) and thus both types of hormone could have a role in production of the exaggerated growth responses in infected leaves and stems. Johnston *et al.* (1974) suggested that as the stimulus for hypertrophy and hyperplasia in peach leaves is not translocated to uninfected areas, the immobile cytokinins rather than the mobile auxins were responsible for this abnormal growth .

Johnston *et al.* (1974) applied their culture supernatant and various combinations of auxins and cytokinins to peach leaves and buds of growing plants but were unable to imitate the symptoms of peach leaf curl disease. Their lack of success was attributed to the inability of the exogenously applied growth hormones to penetrate the bud scales or the cuticles of the leaves (Johnston *et al.* 1974). They suggested that intercellular fungal hyphae of *Taphrina* released cytokinins into surrounding mesophyll cells inside the leaf at a constant rate over a long period.

Asci, which are formed only on the upper leaf surface, perforate the cuticle by both lysis and mechanical action; hyphae growing in the intercellular spaces cause a partial dissolution of the cell walls by secretion of polysaccharide-degrading enzymes, including cellulase (Bassi *et al.* 1984). The exposure of the dense palisade of cylindrical asci give the surface of the lesion a velvety appearance and a whitish grey bloom (Booth 1981), as illustrated in Plate 1. Naked asci, which are unitunicate and have no special apical apparatus for the release of ascospores, release ascospores into the air. The tip of the ascus simply bursts at its thinnest point, responding to a buildup of pressure within the ascus (Syrop 1975a). Fitzpatrick (1934) found that as spores are discharged from the ascus they cling together in a little ball and the tiny spore balls may be blown some distance before breaking up. She also found that when curled leaves were fastened to the guard of an electric fan, the little packets of spores could be caught on slides 2 metres away. However, these spore packets disintegrated immediately they were brought into contact with a drop of water and the 8 ascospores floated apart from each other.

Infected shoots tend to be stunted, somewhat thickened and distorted and light yellow green (Atkinson 1968). Shortening of the internodes crowds the leaves together into rosettes. Many infected shoots are killed. Whilst *T. deformans* is more likely to invade leaves and shoots, blossoms and fruits can also be

attacked, resulting in the development of soft blistered areas on the fruit.

2.2.1 Infection period

The incidence and severity of leaf curl infection depends on the following: the environmental conditions during the susceptible period; the inherent varietal resistance; and past applications of fungicide. Varietal resistance and the effectiveness of some fungicides is discussed in section 2.4.. This section describes environmental conditions conducive to leaf curl infection.

The influence of weather on peach leaf curl disease incidence has been noted by many researchers (Foster *et al.* 1952; Ritchie *et al.* 1981; Tate *et al.* 1987; Fitzpatrick 1935). In short, cold wet summers (13-18°C at bud swell) encourage the disease development and cool, dry springs discourage it.

Foster *et al.* (1952) described the peach leaf curl epidemic of 1951 in Northern South Carolina. During May, some of the severely affected orchards, mostly of the Elberta variety, were nearly defoliated. Factors they believed favoured the development of this epidemic were cool, wet weather during late March and April which was preceded by unseasonally warm weather. It was thought that bud development began before the recommended dormant spray of Bordeaux mix or liquid lime sulphur was applied. Ritchie *et al.* (1981) found that leaf curl was most severe in years when cool, wet weather occurred during bud swell. Fitzpatrick (1935), by means of spraying experiments, closely determined the time of infection in two seasons in Eastern Ontario, and correlated these infections with rain periods. She concluded that leaves were susceptible when emerging from the bud and for a short time after. In New Zealand Tate *et al.* (1985), found that first symptoms of leaf curl were visible on 2 October, 16 days after wet, cool weather and 59 days after leaf bud movement.

Whilst ascospores are discharged in late spring/early summer, little or no infection occurs in late summer. The susceptible period for leaf curl infection is thus short, generally only a matter of weeks. In most seasons new leaves are free from leaf curl. However, in cold, wet summers infection may continue to appear on young leaves for several months (Tate *et al.* 1987).

Fitzpatrick (1935) reasoned that the disappearance of the fungus during summer was because of factors operating after penetration of the leaves had taken place, rather than an inability of the fungus itself to penetrate the leaf. Her observations showed that penetration of leaves occurred readily between temperatures of 13-18°C, well within the normal summer range. Furthermore, conditions under which the plant grows after penetration has occurred materially influence the amount of leaf curl which develops. Those grown at high temperatures develop little or no disease whereas those grown at lower

temperatures become heavily diseased.

New leaves developed so rapidly during summer that their susceptibility period was over before the fungus established an adequate parasitic relationship within the leaf and consequently such infections were unable to develop beyond the initial stages; the fungus dying as the leaf matured (Fitzpatrick 1935). The well defined boundary which always exists between healthy and diseased areas on the mature leaf supports the theory that such a period exists and that later tissues become resistant to any further spread of the fungus. Fitzpatrick (1935) found that this resistance seemed to be correlated with the degree of maturity of the tissue inasmuch as the tip, which matured earliest, was the most resistant part of the leaf.

2.2.2 Late infections

Some researchers (Mix 1935; Tate *et al.* 1987) found late infections of peach leaf curl. Mix (1935) noted that lesions due to late infection were small and in separate areas scattered over the leaf blade, some distance above the base of the season's shoot. He suggested that such lesions also occurred in seasons where early infections occurred, but were not always recognized.

Small curl lesions on leaf blades but no sign of the disease on the petiole or adjacent part of the shoot were observed on leaves arising from the 9th, 10th, 11th and 12th nodes from the base of the shoot (Mix 1935). Peach trees have indeterminate growth and not all the leaves of a season's shoots are present when the bud unfolds in spring. There are usually 6 or 7 leaves present within the dormant bud. Mix's observations of disease on leaves from the 9th and higher nodes is proof of late summer infection of leaf curl.

Late infections, whether primary or secondary, are of little practical significance. Late infections do not severely inhibit tree growth and vigour. Fungicide spray application for peach leaf curl is an attempt at eradication of the fungus, resulting in the destruction of ascospores and budded conidia present on twig and bud surfaces. If carefully done, it controls the disease.

2.3 CULTURE OF *TAPHRINA DEFORMANS*

2.3.1 Isolation of *T. deformans*

(a) From peach leaves

Fitzpatrick (1934) obtained monospore cultures of *T. deformans* by attaching small pieces of curled leaves from which asci were actively releasing spores, to glass slides with vaseline. They were supported above sterile drops of water on sterile slides. After a number of spores had collected in each drop, the spore suspension was streaked onto the surface of clear PDA, with a sterile wire loop. Plates were inverted and examined under the microspore. Single spores, well separated from their neighbours were located and marked. When the colonies produced by the budding of these spores had a 0.5 mm diameter they were picked off with a sterile needle and transferred to slants of PDA in culture tubes.

Cheah and Tate (1986) isolated *T. deformans* by first placing infected portions of leaves onto the lid of an inverted petri dish containing PDA. Ascospores shot upwards and stuck to the agar surface. Single clusters of spores were located with a low power microscope and removed. To prevent contamination from bacteria streptomycin (50 ppm) was added to the PDA media.

(b) From dormant peach buds

To determine if it was possible to isolate *T. deformans* from its overwintering stage Fitzpatrick (1934) initially used artificially inoculated material. A dormant peach seedling was inoculated in January 1932 by painting a water suspension of young culture on the buds. The tree was left outside throughout the experiment. A week later, one of the inoculated buds was removed and crushed in sterile water, loopfuls of which were streaked on PDA. In 24 hours one of the fungi commonly associated with the bud scales of peach was growing. Two days later colonies of *T. deformans* were recognized under the microscope and transferred to PDA slants. Three more isolations of *T. deformans* were made between February and April, confirming that sprout conidia can survive winter conditions whilst on the surface of peach buds.

Fitzpatrick (1934) then isolated *T. deformans* from trees affected by a heavy infestation of *T. deformans* in the previous season and which had not been subsequently sprayed with fungicide. Twenty plates of PDA were streaked with the washings from buds of these trees and parallel plates were streaked from those of artificially inoculated ones. This work strongly supported the claim that spores lodged on the surface of the trees are responsible for the persistence of leaf curl from season to season.

Mix (1924) was not able to isolate the leaf curl fungus from twig surfaces because of contamination

from budding and filamentous fungus found on twig surfaces, all of which grew faster in culture than *T. deformans*. On any medium Mix found to support *T. deformans* growth, including an acid medium with pH 3.5, saprophytic forms grew as well or better.

2.3.2 Growth in culture

In culture, all species of *Taphrina* develop budding yeast-like colonies that range from a salmon-pink to pinkish -yellow and have a characteristic dry, yeast-like appearance on the colony surface (Kramer 1973). Fitzpatrick (1934) described the colonies as convex, shiny, butyrous and white in colour when young, becoming pink with age.

Although it is always the saprophytic stage that develops *in vitro* these blastosporic cells are capable of infecting a host (Booth 1981). In culture ascospores tend to produce a series of budded secondary spores rather than a true mycelium (Martin 1925), but they may also produce short infection hyphae. Secondary spores, in turn, continue to grow by budding when on artificial media, but on a host plant they produce infection hyphae that develop into true mycelium.

Thick-walled spores like those described by Mix (1924) and by Martin (1925) are found in all old cultures. They are numerous after two weeks on media favourable to *T. deformans* growth. These thick-walled spores represent a dormant stage of the fungus. They germinate to produce either a series of thin-walled spores or short hyphae (Martin 1940). Asci are 25-50 µm by 8-11 µm, club-shaped, unitunicate with a flattened top which splits on discharge (Booth 1921).

2.3.3 Environmental conditions

Mix (1924) found optimum growth of *T. deformans* on PDA media at temperatures just below 20°C; the minimum temperature for growth was below 10°C and the maximum, 26-30°C. The growth of *T. deformans* in culture is relatively slow given its yeast-like appearance; it can take 4 days at 20°C before growth is readily seen with the naked eye.

Mix (1924) demonstrated that sprout conidia dried on glass cover strips remained alive for 140 days at 30°C and 315 days at lower temperatures. It is not known what humidity was used.

Martin (1940) grew cultures of *T. deformans* at temperatures of 4°, 12°, 16°, and 20°C and found temperatures from 12° to 16°C were especially favourable for both "budding" and the formation of germ

tubes.

2.3.4 Media

T. deformans thrives in culture on a wide range of media, many with a low food content such as a peach gum dilute solution (Mix 1935). Media used for the culture of *T. deformans* by Martin (1940) included 2% dextrose broth and 0.1% tryptophane and 1% peptone. Fitzpatrick (1934) thought that *T. deformans* had better growth in soft agar media. PDMA + peptone, PDA + peptone and Gibco PDA + peptone were found by Cheah and Tate (1986) to promote significantly better growth than PDMA, PDA and nutrient media. Media recipes are given in Appendix C.

T. deformans spores will bud readily in broth media of various sorts but the heaviest growth is on media containing a vegetable extract, such as from potato, as a base (Fitzpatrick 1934). The function of the potato extract is thought to be entirely a nutritive one. In broth culture growth is entirely sedimentary and the liquid remains clear (Fitzpatrick 1934).

Martin (1940) grew cultures of *T. deformans* on various kinds of media (pH3.5-5). Media favourable for both budding and the formation of germ tubes were a 2 per cent dextrose broth with 0.1% tryptophane, and also the same dextrose broth with 1% peptone. Similar results were obtained with potato-dextrose agar.

2.3.5 Summary

T. deformans was isolated by placing infected sections of leaves (showing the fungal bloom) onto lids of inverted petri dishes, beneath PDA. Ascospores shoot upwards and stick to the agar surface within 24 hours. Isolation of *T. deformans* from dormant winter buds is possible but has been difficult for many researchers because of contamination from saprophytic fungi. In culture *Taphrina* species develop the saprophytic stage only. These budding yeast-like colonies range from pink to yellow and have a characteristic dry appearance on the colony surface. They are capable of infecting a host. *T. deformans* can grow on a range of media. Researchers in recent years have tended to use commercial preparations of PDA media with the addition of 1% peptone. This media is simple to prepare and use and the peach leaf curl fungus grows adequately on it. Temperatures between 12°C and 16°C were most favourable for the budding of *T. deformans* and the formation of germ tubes.

2.4 CONTROL OF *TAPHRINA DEFORMANS*

Introduction

On unsprayed trees peach leaf curl can become a serious disease. It may destroy the first leaves and much of the crop year after year, gradually weakening the tree until it dies or is invaded by other organisms. It is particularly important to control the disease in the establishment of an orchard when cashflow is at a minimum and a good fruit yield is essential for an early return on investment.

The biology and lifecycle of the peach leaf curl fungus and its effect on susceptible peach and nectarine hosts was described in section 2.2. It was established that the fungus overwintered as ascospores in the bud scales of the host. In spring overwintering spores are rain-splashed onto exposed developing leaves and infection of the peach host occurs. Control by fungicide spray is aimed at destroying conidia present on twig and bud surfaces prior to their penetration of host tissue.

Since growers have achieved effective and cheap control of peach leaf curl with Bordeaux mixture there has been minimal research in the last fifty years for other control methods. New Zealand researchers, Tate *et al.* (1989 unpublished) found that although one well timed spray in early spring could completely control the disease, in practice several sprays from leaf fall until September may not completely prevent it. Once established, the fungus could spread to newly emerging leaves whenever cool wet weather occurred.

Control failure was considered a function of incorrect spray timing and/or incomplete coverage of spray by Tate *et al.* (1987). Claims by Hawkes Bay growers in 1987 of fungal resistance were not confirmed until 1989 when Cheah and Tate (Anon 1989) isolated a strain of *T. deformans* with low sensitivity to copper fungicide in laboratory tests. Tests are underway to confirm their findings.

Several fungicides have been effective in controlling leaf curl overseas (Atkinson 1968) and there are anecdotal reports of other control methods. For example it has been suggested that soil dressings of magnesium sulphate (Epsom salts) control leaf curl and that seaweed extracts also control the disease. No proof of these control measures has been found in the scientific literature.

It is possible that the characteristic shedding of the first formed infected leaves, and subsequent growth of a second lot of healthy foliage has led some to believe that the particular 'remedy' they applied early in the season controlled the disease later in the season. This may be the situation with seaweed extracts which to date have had anecdotal reports of success for the control of peach leaf curl.

This section describes control measures for leaf curl. It is divided into four parts:

2.4.1 varietal resistance

2.4.2 fungicides

2.4.3 seaweed extracts and

2.4.4 other controls

2.4.1 Varietal resistance

Since 1906 when Rivers noted the first case of peach leaf curl resistance (Janick and Moore 1975), very little effort has gone into breeding for resistance to this disease, probably because it has been controlled easily in the past with a single copper spray at bud burst. Vol'vach (1986) commented that *T. deformans* was a serious problem in all regions of commercial peach growing in the USSR and that whilst chemical control could be effective, the use of resistant cultivars was economically and ecologically more desirable. Hence, there was a predominance of literature on peach leaf curl resistance from the Eastern block countries; USSR, Czechoslovakia, Hungary, Romania, Yugoslavia and Bulgaria where the few breeding programmes for resistance to leaf curl were. This made the review of literature on peach leaf curl resistance very difficult and much of the following information was taken from English abstracts rather than full reports of the research.

The few reports on the susceptibility of peach and nectarine cultivars to *T. deformans* (Ackerman 1953, Foster and Petersen 1952, Lorenz 1976b, Ritchie and Werner 1981) verify a difference between cultivars in their susceptibility to peach leaf curl. It is not known if there is a genetic base for this resistance. Janick and Moore (1975) suggest a polygenic system of control for susceptibility to peach leaf curl but there has not been further research on this point.

Ritchie and Werner (1981), analysised progeny of 862 seedlings synthesized from 13 matings and found that leaf curl susceptibility was heritable. In general, Redhaven and most cultivars derived from Redhaven were tolerant to leaf curl, whilst Redskin and those derived from Redskin were susceptible or highly susceptible. Their analysis was supported by observations in growers' orchards and by Foster and Petersen (1952). In contrast to Ritchie and Werner's findings, Koch (1984) found that Redhaven peaches were susceptible to leaf curl, especially in rainy years.

The heritability of peach leaf curl was confirmed by Fideghelli *et al.* (1983) in an analysis of open and cross pollinated seedlings from and immune cultivar, PC408 which was compared with the susceptibility rating of 43 peach and 59 nectarine cultivars. On a score of 0-5 (0, no disease) PC408 and derived seedlings were always 0; whilst under the same conditions the average score of the peach and nectarine cultivars was 4.4.

The suggestion that cultivars which leaf out and bloom early might be more susceptible to peach leaf curl than cultivars that initate growth later because they escape the early cool, wet weather most conducive to infection was discounted with Ritchie and Werner's data (1981). They found that

susceptible varieties bloomed in both early and late periods. This suggests that factors other than time of bloom are involved in susceptibility to leaf curl.

Table 2.1 summarises the susceptibility of 167 peach and nectarine cultivars' to leaf curl reported in the literature. Peach and nectarine varieties are grouped into those of low (63 cultivars), moderate (72 cultivars) and high (44 cultivars) susceptibility to leaf curl, as indicated by the researchers. In most cases it is unclear what criteria were used by researchers to assess the degree of susceptibility to leaf curl because complete research reports were not able to be obtained. It is also possible that terminology may have been confused in the translation to the English abstracts. For example, immune may have been translated for high tolerance.

Table 2.1 shows that of the literature studied, 63 varieties of peaches and nectarines had low susceptibility or high tolerance to leaf curl and some researchers (Zagorodnaya 1985, Fideghelli *et al.* 1985) believed certain cultivars were immune.

Only 14 of the 167 results for cultivars summarised in Table 2.1 were studied in more than one environment and 9 of these were inconsistent: Elberta, Amsden, Redhaven, Early Red Fre, Nectared, Loring, Redskin, Rio Oso Gem and Sunhigh. The largest discrepancies were with Loring (found highly susceptible by Ritchie and Werner (1981) but of low susceptibility by Foster and Petersen (1952)), and Elberta which had low susceptibility by Alexandri and Filip (1977) in Romania, moderate by Ritchie and Werner (1981) in North Carolina and high by Foster and Petersen (1952) in South Carolina. Redhaven, Amsden and Early Red Fre had low-moderate susceptibility and Nectared, Redskin, Rio Oso Gem and Sunhigh had moderate to high susceptibility.

Ritchie and Werner (1981) in a comprehensive study of 67 peach and 11 nectarine cultivars, found a large range of symptom severity between varieties and interpreted this as susceptibility differences between varieties. They observed that symptoms on the more tolerant peach cultivars such as Redhaven and Clayton were limited to the earliest emerging leaves and leaves on tolerant cultivars did not exhibit the typical hypertrophic symptoms of leaf thickening, puckering, and curling. The predominant symptom was the development of a yellow to red discolouration on only the earliest emerging leaves and subsequent leaf drop.

TABLE 2.1

Susceptibility of 167 Varieties of Peach and Nectarine to Leaf Curl

Superscripts denote the reference which are listed in Appendix A. Where susceptibility to leaf curl was found different by different researchers, this is indicated in the table with the appropriate letter; 'l' low; 'm' moderate and 'h', high susceptibility.

LOW (tolerant)	MODERATE	HIGH (very susceptible)
Alexander Early[12]	Andross[4]	Afterglow[2]
Ambergem[2,1]	Belle of Georgia [2,4]	Autumn Gem[4]
Amsden[3]; 8m,12m	Best May[2]	Babygold[4.1]
Candor[4]	Blake[4]	Biscoe[4]
Capucci[1]	Burbank Elberta[2]	Camden[4]
Cardinal[1]	Carson[4.1]	Cary Mac[4]
Cherryred[2]	Cresthaven[9]	Colomba-Rubiette[11]
Clayton[4]	Derby[4]	Early Elberta[12]
Com-pact Redhaven[4]	Dixired[2,4.1]	Early Sungrand[4.1]
Correll[4]	Dixon[4]	Elberta Late [12]
Davidson Red leaf[2]	Early fair beauty[2]	Firebrite[4]
Desirable[2]	Early Halehaven[2]	Flamecrest[4.1]
Dixigem[2]	Early Red Fre[2.2l]	Flavorcrest[4]
Dixiland[4]	Early Triogem[2]	Gemmer's Late Elberta[2]
Early Elberta[2]	Emery[4]	Goldeneast[2]
Early Jublilee[2]	Everts[4]	Junegold[4]
Early Red[5]	Fairtime[4]	Late Legrand[4.1]
Early Redhaven[4, 5]	False Sunhigh[2]	Lizzie[2]
Early Vedette[2]	Fayette[9]	Loring[4;2l]
Elberta[1]; 4m 2h 12h	Fertile Hale[2]	Norman[4]
Ellerbe[4]	Fireglow[2]	Redglobe[4]
Elma[3]	Fisher[2]	Redskin[4;2m]
Eriogen[1]	Fort Valley 110[2]	Regina[4.1]
Fairhaven[2]	Fort Valley 993[2]	Rio Oso Gem[2;4m]
Fay Elberta[2]	Frant[8]	Springcrest[4.1]
Fort Valley [2]	Gem[4]	Stark [12]
Gold Mine[1]	Glohaven[9]	Starlite[4.1]
Golden Beauty[2]	Golden Globe[2]	Sullivan Early Elberta[2]
Halberta[2]	Halegold[2]	Summerset[4.1]
Halehaven[2]	Hamlet[4]	Sunbeam [12]
Imperio[1]	Harvester[4]	Sunbrite [4]
Indian Blood[2]	Jefferson[4.1]	Suncrest [4.1]
J.H. Hale[2]	July Elberta[10,2]	Sunhigh [4;2l]
Kirkman Gem[4]	Kudesnik[8]	Troy[4]
Konservynyi Rannii[6]	Lebedev[8]	Windsor[4]
Magoscorlup[1]	Lovell[4]	
Merrill June[2]	Madeleine Pouvet[10.1]	
Missouri[2]	Marsun[4.1]	
Nectar[2]	Maygold[10]	
Nectared-2[5]	McNeely[4]	
Newday[2]	Monroe[4]	
Ozark[2]	Novelroe[4.1]	
Pekin[4]	Othelle[10]	

LOW (tolerant)	MODERATE	HIGH (very susceptible)
Prairie Dawn[2]	Pocahontas[10.1]	
Proskauer[3]	Podarok Kryma[8]	
Ranii Kubani[7]	Ranger[4]	
Raritan Rose[2]	Redwin[10.2]	
Redcrest[2]	Reliance[4.1]	
Redhaven[4,2; 3m,12m]	Sentinel[9]	
Rubired[4]	Sieger[3]	
South Haven 20[2]	SouthHaven[12]	
Southern Glow[2]	Southland[4.1]	
Springtime[5]	Springbrite[4]	
Stark Redgold[5]	Starking Delicious[2]	
Summer Crest[2]	Sunday-Elberta[2]	
Toss Kino[1]	Sunhaven[3,5]	
Triogem[2.1h]	Sunqueen[4]	
Vivid[4]	Sunshine[10.2]	
Vorgebirgsfirsich[3]	Triumph[12]	
	Velvet[4]	
	Washington[12]	
	Whynot[4]	
	Winblo[4]	
Nectarines		
Mericrest[13]	Colombia[4]	Earliblaze[4.1]
	Harko[4.1]	Fantasia[4.1]
	Red Chief[10]	Flamekist[4.1]
	Nectared 4[4;5h]	Flavortop[4.1]
		Garden State[2]
		Nectared 7[4.1]
		Rose[4.1]

Ritchie and Werner (1981) found that whilst susceptibility varied greatly between cultivars, none was immune. However, Fideghelli *et al.* (1983) found that a chance seedling from Central Italy was immune to leaf curl and did not show any leaf curl symptoms between 1974 and 1982, even under conditions of severe natural infection. The clingstone fruit however was of poor firmness and quality.

Under conditions of "severe natural infection" in the Crimea, Vol'vach (1986) found high resistance in Early Redhaven, Springtime, Nectared4, Sunhaven, Stark Redgold, Nectared2 and Early Red. Tavdumadze (1971) noted 'relative resistance' in several Russian cultivars of peach and one nectarine.

Some researchers (Zagorodnaya 1985, Grigorov 1985, Simakina 1980, Nitransky 1983, Ritchie and Werner 1981) studied combined breeding factors of disease resistance to fungal diseases and commercial potential in terms of yield. Where low susceptibility or immunity to peach leaf curl was found this was not always followed with a recommendation for commercial use (Nitransky 1982; Zagorodnaya 1985).

Fideghelli *et al.* (1983) are using an immune variety (named PC408) to investigate the genetic background of the leaf curl resistance character and to breed resistant varieties of market quality. They also plan to study the correlation between resistance to leaf curl and that to *Pseudomonas prunus* and other serious diseases of stonefruit.

Nitransky (1982) found that none of the 87 peach and nectarine cultivars evaluated over five years were resistant to *T. deformans* but Madeleine Pouyet and Pocahontas had the best "relative resistance". However these were not recommended for commercial cultivation.

Ritchie and Werner (1981) found that in general the cultivars most tolerant to leaf curl were also the most tolerant to bacterial leaf spot (caused by *Xanthomonas pruni*). Clayton *et al.* (1976) found a positive correlation between leaf curl resistance and less susceptibility to flesh browning. The highest combined resistance to disease was found in Earligold, Springcrest, Merill Gem Free, Sentinel and Armgold by Nitransky (1983). Unfortunately this aspect was not considered by all researchers.

Ryabova (1980), in USSR, found that whilst none of the 100 peach cultivars studied were immune to *T. deformans* some cultivars had ten percent or less leaf infection. Similarly, in the Crimea, Simakina (1980) found that some varieties were resistant to peach aphid, *Myzus persicae* and to peach leaf curl, *T. deformans*. Ranii Kubani described as an early cultivar of vigorous growth which yielded an annual average of 29 kg/tree over ten years had tolerance to *T. deformans*. Simeone (1984), in Italy, found that 46 peach and 62 nectarine cultivars were in general highly susceptible to all pathogens.

Nitransky (1983), field evaluated 29 two year old peach and nectarine cultivars for three fungal diseases: leaf curl, powdery mildew and *Clasterosporium carpophila*. Sunshine and Redwin were the most resistant to *T. deformans* whilst Earligold, Springcrest, Merill Gem Free, Sentinel and Armgold had the highest combined resistance. Nitransky (1974) found better than average resistance to *T. deformans* in the peaches Maygold, Othelle, July Elberta and Red Chief nectarine.

In Yugoslavia Todorovic & Misic (1982) found that Fayette, Cresthaven, Glohaven and Sentinel were the least susceptible of 11 peach cultivars, 15 peach x nectarine, peach x peach and nectarine x nectarine hybrids to leaf curl. They also found that a high proportion of hybrids (25-50%) with Cresthaven peach or Silverhode nectarine parentage were NOT infected with peach leaf curl. Nitransky (1982) also found Cresthaven had commercial promise.

Romanian researchers Alexandri and Filip (1977) tested 85 peach cultivars for resistance to *Sphaerotheca pannosa* var. *persicae* and *T. deformans* in trials over 3 to 6 years. Cardinal, Capucci 6, Gold Mine, Magoscorlup, Eriogen, Toss Kino, Imperio and Elberta were resistant to both fungi. These varieties are not known in New Zealand but Elberta has been commonly studied in the USA (eg Ritchie *et al.* 1981; Foster *et al.* 1952).

Ritchie *et al.* (1981) suggested from their study of eleven nectarine cultivars and 67 peach, that nectarines were more susceptible to leaf curl than peaches. This however contrasts the work of Ackerman (1953), who concluded the opposite (ie nectarines are less likely than peaches to be severely infected by *T. deformans*). The small number of nectarine cultivars studied by Ritchie and Werner prohibits any conclusive statement. Smith *et al.* (1988) also found differences in susceptibility between peach and nectarine cultivars, and in particular the semi-wild cultivars known in France as "pechers de vigne" were very resistant.

Another uncertainty in interpreting Ritchie and Werner's (1981) data was that 51 peach and four nectarine cultivars had been sprayed in the previous growing season with wettable sulphur for the shuck-fall and cover sprays, and benomyl and Captan^{R} in the pre-harvest sprays. Northover (1978) reported that pre-harvest sprays of Captan^{R} could result in the control of leaf curl in the following spring. It is thus probable that these fungicide sprays affected Ritchie and Werner's results. None of the 16 peach cultivars which had not had a fungicide application in the previous season had low susceptibility to leaf curl, although seven had only moderate susceptibility. Two of the nectarine cultivars, Nectared 6 and Lafayette, had no symptoms of leaf curl but Ritchie *et al.* (1981) attributed this more to the fungicide applications of the previous season than varietal resistance. The 12 varieties of peach with low susceptibility in Ritchie and Werner's data had all had fungicide applications in the previous season.

These are noted in Table 2.1 with the superscript 4. Those which did not have fungicide in the previous season have superscript 4.1.

Harrisons Trees of Palmerston North in New Zealand sell two peach varieties as leaf curl resistant; cvs. Gordon Glory and Winiata Gold. Gordon Glory, a clingstone peach with similar flesh and flavour to Golden Queen ripens in March/early April and has yellow skin with a bright orange-scarlet cheek. Gordon Glory is thought to have originated in Palmerston North. Winiata Gold is also similar to Golden Queen but has softer flesh. It ripens in mid-March and is believed to come from a Taihape selection (Harrison 1988). In the 1989 season in Palmerston North, fruit from three year old trees of both these cultivars ripened in early February. Data on the susceptibility of these cultivars to peach leaf curl has been examined in section 4.

Increased resistance to peach leaf curl is thought to occur if peaches are raised on their own roots by softwood cuttings (Weber 1972). Weber claims this also reduces the incidence of fungal diseases which attack grafted wood.

2.4.1.1 Methods used

As most of the research into susceptible varieties was done in Eastern Europe it was difficult to obtain information on the methods used by these researchers as these were generally not detailed in the abstract literature.

Ritchie and Werner (1981) rated leaf curl susceptibility on a scale of 0 (no symptoms) to 9 (the entire leaf extensively thickened, puckered and curled, making it two or three times the size of an uninfected leaf). In table 2.1 scores averaging 0-2 were classed as low susceptibility to peach leaf curl; 2-5, moderate and 6-9 high. The scale rated the amount of infection as a percentage of leaves with symptoms. Trees were rated about four weeks after full bloom. Between two and 65 trees per cultivar were observed and many cultivars were located in several fields over an area of 200 ha. The entire tree was observed; affected leaves were given an average rating and the percent of leaves infected was estimated.

Foster and Petersen (1952) rated 73 varieties of peach and nectarine for susceptibility to leaf curl on a scale of 0 to 5. These have been interpreted in Table 2.1 as: 0-1 low susceptibiltiy; 2-3 moderate susceptibility and 4-5 high susceptibility. No variety observed was immune. Nesic *et al.* (1971) divided varieties into relatively resistant, moderately susceptible and very suceptible.

2.4.1.2 Summary

There have been few breeding programmes for resistance to peach leaf curl because peach leaf curl has been controlled easily with a single copper spray at bud burst. Of the few programmes cited in the literature most were from the Eastern block countries; USSR, Czechoslovakia, Hungary, Romania, Yugoslavia and Bulgaria. The reason for their emphasis on breeding for resistance is perhaps best summarised by Vol'vach (1986) who wrote that *T. deformans* was a serious problem in all regions of commercial peach growing in the USSR and whilst chemical control could be effective, the use of resistant cultivars was economically and ecologically more desirable.

There is a difference between cultivars in their susceptibility to peach leaf curl (Ackerman 1953, Foster and Petersen 1952, Lorenz 1976b, Ritchie and Werner 1981) but there are inconsistencies in the results of cultivar susceptibility under different environmental conditions. Nine of the 14 cultivars (summarised in Table 2.1) studied under different environmental conditions had inconsistent results. Redhaven, Amsden and Early Red Fre had low-moderate susceptibility and Nectared, Redskin, Rio Oso Gem and Sunhigh had moderate to high susceptibility. The rating for cultivars Loring and Elberta was more variable and ranged from low to high susceptibility. There are conflicting results on the difference between nectarine and peaches in their susceptibility to peach leaf curl and hence no conclusion can be drawn. Given such vagaries in the reported literature and the few cultivars actually studied under different environmental conditions it would be difficult to list with confidence the susceptibility of cultivars to peach leaf curl.

However, "immunity", "high resistance", "relative resistance" "not more than 10% infection" were noted by some researchers (Vol'vach 1986; Tavdumadze 1971; Fideghelli *et al.* 1983; Nitransky 1982; Ryabova 1980) for certain varieties and 63 of the 167 cultivars listed in Table 2.1 had low susceptibility or tolerance to leaf curl. Cresthaven and a high percentage of Cresthaven hybrids were found to have commercial promise under two different environments (Todorovic & Misic, 1982; Nitransky, 1982).

It is not known if there is a genetic base for this resistance but it has been suggested that a polygenic system of control does exist (Janick and Moore 1975). Further research is required. The heritability of peach leaf curl was confirmed by Fideghelli *et al.* (1985) and Ritchie and Werner (1981). The latter also confirmed that factors other than time of bloom are involved in susceptibility to leaf curl as susceptible varieties bloomed in both early and late periods.

In many cases it is unclear what criteria were used by researchers to assess the degree of susceptibility to leaf curl because complete research reports were not available. However, in general, leaves were scored

for percent disease on a scale of 0-5.

Breeding programmes have generally considered cultivars with combined resistance to diseases and with useful yield potential. However, low susceptibility or immunity to peach leaf curl did not necessarily imply a recommendation for commercial use of the cultivar. Nitransky (1983) found Earligold, Springcrest, Merill Gem Free, Sentinel and Armgold cultivars had the highest combined resistance to diseases and recommended these for futher breeding programmes. Unfortunately there were few reports of combined resistance in the reports of breeding programmes studied. Correlation was found between cultivars tolerant to peach leaf curl and bacterial spot, caused by *Xanthomonas pruni* (Ritchie and Werner 1981); and less susceptibility to flesh browning (Clayton *et al.* 1976).

2.4.2 Fungicides

The role of fungicides in the control of peach leaf curl is to kill spores present on the trees, especially those germinating, and to have enough chemical present on the buds for rain to wash some of the fungicide in between the bud scales and kill any spores lodged there (Heyns 1965). Once the fungus has entered the leaves no chemical yet discovered will control it, so treatments must be applied before leaves emerge. However, there has been some laboratory success with the use of EBI systemic fungicides (Tate *et al.* 1989) as an eradicant of peach leaf curl.

The main factors which govern the efficiency of a spray in a given season are:
i) the thoroughness with which the spray is applied and
ii) the time between its application and the period favourable to infection.

The thoroughness of spray application is directly related to the equipment efficacy, weather conditions and experience of the spray applicator. Poor results with the application of fungicides may also be because growers may use less than the recommended rate and make applications under adverse weather conditions (Northover 1978). Information on these factors is readily available to growers and will not be discussed except for the actual timing of spray applications.

2.4.2.1 Timing of fungicides

Whilst many researchers indicate that the best time to spray is bud burst, the vagaries of climate and of biological systems imply that this may not always be the most effective time. Section 2.2 showed that there are two main periods in the lifecycle of the leaf curl fungus where it is susceptible to fungicides:

leaf fall, as ascospores produce yeast-like colonies of overwintering spores; and at the beginning of bud swell in spring, when overwintering spores become physiologically active active and germinate. The effectiveness of different fungicides and their time of application (where documented) is summarised in Table 2.2 and details given in the text.

Penetration of leaves by leaf curl fungi can occur at temperatures between 10°C and 21°C (50 - 70°F), with optimum temperatures between 13°C and 18°C (50 - 60°F) (Fitzpatrick 1935). A high proportion of diseased shoots can become heavily diseased, with all the successively unfolding leaves affected for as long as cool, wet conditions in spring persist.

The parasitic phase of leaf curl is presumed to be prevented mainly by killing the inoculum on twigs and bud scales, rather than by protecting the vulnerable young leaves. Thus the severity of leaf curl could be substantially reduced by using specific fungicides in the previous growing season, in addition to any treatments to dormant trees.

It has been common commercial practice to use two sprays, one as the buds start to move and another a week later, to ensure that timing is right and that full coverage is obtained (Atkinson 1968). Other practices have been to spray once in the autumn or winter and again just before leaf bud burst (Booth 1981), as leaf buds started to swell. Autumn sprays themselves may not be adequate in winter rainfall areas (Heyns 1965), but an autumn application of a copper containing compound in addition to the spring spray gives better control (Booth 1981). Heavy rains wash away fungicides and spread fresh inoculum from unsprayed trees.

Despite regular spraying to control leaf curl in Hawke's Bay, a major stonefruit growing area of New Zealand, leaf curl became established each season (Brier 1988). A new pattern emerged where the first leaves were healthy, but subsequent leaves became diseased. Initial laboratory tests in 1987 for fungicide resistance were negative and monitoring of the infection periods in Hawke's Bay suggested that a late strain was not responsible. Instead, growers were told that their bud movement spray was earlier than the infection period and so not providing adequate control. Tate (pers comm. 1988) believed that the infection period for Fantasia nectarines occurred later than normal in the 1986 and 1987 seasons - at the end of August in 1987 and mid-September in 1986. This meant that the traditional timing of sprays in July/early August was 4-6 weeks too early.

However, as has already been mentioned, a strain of *T. deformans* resistant to copper fungicides was isolated in 1989 by Cheah and Tate (Anon 1989).

2.4.2.2 Fungicides used

Commercially, control of leaf curl is considered adequate when less than 2% of the shoots are diseased (Tate *et al.* 1985). Table 2.2 summarises the effectiveness of different fungicides for the control of peach leaf curl as documented in the literature.

For many years the standard control for leaf curl was Bordeaux mixture at a strength of 6:8:100. This gave uniformly good results when applied at bud movement (Atkinson 1968), but before buds opened. More recently, fixed copper fungicides (cupric hydroxide and copper oxychloride) have become popular because they are easier to use and handle than Bordeaux (Tate *et al.* 1985).

Lime sulphur has been used: it gave satisfactory peach leaf curl control in South Africa (Heyns 1965), in spring applications in Czechoslovakia (Seidl *et al.* 1976) and in India (Bose *et al.* 1972). Peach leaves are not as easily scorched by copper as by lime sulphur (Matthee *et al.* 1975). Lime sulphur can damage shoots where the past season's growth is still immature and thin (Heyns 1965). The advent of fungicides with low phytotoxicity made lime sulphur less popular.

Replacements for Bordeaux have been, and still are, actively sought. Some Pthalimides and Dithiocarbamates proved as effective as the copper fungicides, with less phytotoxicity but did not control bacterial diseases such as stonefruit blast, bacterial canker or gumspot, like the coppers do. In New Zealand, where up to five Bordeaux or fixed-copper sprays can be applied between autumn and late dormancy to control bacterial diseases, the additional use of sprays such as captafol to control peach leaf curl is not justified (Tate *et al.* 1985).

TABLE 2.2 EFFECTIVENESS OF SOME FUNGICIDES AGAINST PEACH LEAF CURL

FUNGICIDE	REFERENCE	APPLICATION TIME	EFFECTIVENESS
COPPER			
Bordeaux	Sproule 1978	bud movement	As effective as ziram, ferbam, captafol etc
	Atkinson 1968		Good
	Tate *et al.* 1985		Good
Cupric hydroxide	Tucker *et al.* 1984	autumn	More effective than chlorothalonil or lime S
		aut. and delayed	
		dormant	No difference between application times
		delayed dormant	
Copper oxychloride	Booth 1981	autumn	Phytotoxic
		spring	Better than autumn
	Matthee *et al.* 1975	autumn	More effective than spring. Best control if combined with a thiram spray 3 weeks before bud swell.
	Wilson 1937	autumn	Effective for next season's control.
Copper sulphate & lime sulphur	Yoder *et al.* 1984	dormant (early Jan)	Not adequate
SULPHUR			
Lime sulphur	Heyns 1965		8l/100l -"Satisfactory control"
	Bose *et al.* 1972	before bud burst & after full bloom (1:15 & 1:40)	As effective as benomyl or Captan
	Yoder *et al.* 1984	early spring	Acceptable control, even though heavy inoculum from previous season
	Seidl *et al.* 1976	bud break	Satisfactory
PTHALIMIDES			
Captan	Bose *et al.* 1972	0.075% ai	as effective as benomyl or lime sulphur
	Zehr *et al.* 1983		Effective when applied with Funginex
	Smolyakova 1977	spring (May)	Better control than zineb, Bordeaux, benomyl
		& Sulphur	Minimum phytotoxicity
	Seidl *et al.* 1976	bud break	Satisfactory
	English 1958		Less effective than Ziram or $CuSO_4$
	Altinyay *et al.* 1978	50% leaf fall & 10 days later	300g/100l **not effective**
	Zehr *et al.* 1983	after bloom	**No effect** on mature Redhaven peach after one spray
	Northover 1978		Less effective than Ferbam or captafol in cool, wet conditions
Captafol	Burr *et al.* 1984	autumn	Very good, even with 45% infection in controls
	Sproule 1978	spring	Good control
	Northover 1978	before or after leaf fall	240g/100l more effective than Ferbam
	Tate *et al.* 1985	before bud swell; 1 or 2 sprays 7 days apart	200g/100l near complete control
	Tate *et al.* 1985	as above	100g/100l as effective as Bordeaux. More effective than $CuSO_4$.
DITHIOCARBAMATES			
Ziram	English 1958	late autumn	Very effective. More effective than $CuSO_4$ & equal to Bordeaux

	Sproule 1978		Good control
Ferbam	English 1958	late autumn	Very effective
	Burr *et al.* 1984	autumn only	Very effective, even with 45% infection in controls
	Tucker *et al.* 1984	autumn only	11% leaves curled
		aut. & delayed dormant	2% leaves curled
		delyd. dormant only	7% leaves curled
			Control 72% curled
			More effective than chlorothalonil or Lime S
	Yoder *et al.* 1984	early spring	Good control even though heavy inoculum previous season
	Drake 1984	dormant late autumn	Fair control on Redhaven, Newhaven, Mericrest
Mancozeb	Seidl *et al.* 1976	bud break	Satisfactory control
ERGOSTEROL BIOSYNTHESIS INHIBITORS (EBI)			
Prochloraz	Tate *et al.* unpub	leaf bud movement	Less effective than chlorothalonil or copper hydroxide
Propiconazole	1989	85% leaf fall	Control 52% curl; EBI 20-44% curl; Protectants 0-3% curl
Myclobutanil		blossom	
QUINONES			
Dichlone		early spring	Acceptable control even though previous season had heavy inoculum
	Zehr *et al.* 1983		Good control of mature Blake peach
PYRIMIDINES			
Fenarimol	Zehr *et al.* 1983		Inadequate control of Blake peach
NITRILES			
chlorothalonil	Zehr *et al.* 1983	bloom	Almost complete control of Redskin & Redhaven peach
	Burr *et al.* 1984	autumn & spring	Complete control of mature trees
	Burr *et al.* 1984	autumn	Very effective even under heavy inoculum
	Tucker *et al.* 1984	autumn	Less effective than Ferbam or copper hydroxide
		aut. & delayed dormant	Severe curl due to cool wet spring
		delayed dormant	
	Drake 1984	dormant spring	Better than dormant late applications of chlorothalonil, Ferbam or basic copper
	Tate *et al.* unpub. 1989	leaf fall	Control 52% curl; Treated 0-3% curl
		leaf bud movement	
		bloom	
BENZIMIDAZOLE			
benomyl	Bose *et al.* 1972		0.075% ai as effective as captan or lime S
OTHER			
Off-shoot T	Burchill *et al.* 1976	2 winter sprays	5% a.i. Effective control
chlorothalonil & cupric hydroxide	Tate *et al.* unpub. 1989	85% leaf fall	Disease reduced to 2%

The Pthalimides Captan^R and Captafol^R have given effective control of leaf curl (Bose *et al.* 1972; Zehr *et al.* 1983; Smolyakova 1977 -spring application; Seidl *et al.* 1976 -spring; Northover 1978; English 1958; Burr *et al.* 1984; Sproule 1978; Tate *et al.* 1985). However, not all researchers found Captan^R effective on peach leaf curl. Altinyay *et al.* (1978) found that at a rate of 300g/100l it did not control leaf curl when applied at 50-60% petal fall and 10 days later, in addition to, or instead of, early spring spraying. It also had no effect when applied as a single spray on mature Redhaven peach trees after bloom (Zehr *et al.* 1983). Northover (1978) found that Captan^R was less active under conditions favouring leaf curl incidence (ie cool and wet) than the fungicides Captafol^R or Ferbam^R.

Single spring applications of Captafol^R gave good control of peach leaf curl (Sproule 1978). Captafol^R was more effective than Ferbam^R when applied either before or after autumn leaf fall (Northover 1978) and was more persistent on bark than Ferbam^R, Captan^R or Bravo^R. At twice the recommended rate, ie 200g/100l, it gave better control of leaf curl than other fungicides (Tate *et al.* 1985) in New Zealand. This rate for captafol^R was slightly above the recommended label rate (80-160g/100l) but less than that used by Northover (240g/100l) in his 1978 trials. Tate *et al.* (1985) found virtually complete control of leaf curl by one, or at the most, two well-timed sprays 7-10 days apart, starting immediately before bud swell. Californian official recommendations omit the bud swell sprays completely if one post-leaf fall application has been made. Captafol^R is not allowed on crops grown for export because of residue restrictions imposed by importing countries.

The dithiocarbamates such as Ferbam^R and Ziram^R introduced in California, after research by English (1958), avoided the phytotoxicity problems of autumn copper sprays. Late autumn applications were found by English (1958), Burr and Smith (1984), Drake (1984) and Sproule (1978) to be effective in controlling leaf curl. English found them as effective as Bordeaux and significantly better than copper sulphate sprays. Tucker *et al.* (1984) established that autumn and delayed dormant applications of Ferbam^R were more effective in controlling leaf curl than Lime sulphur or Bravo^R. However, early spring applications have also been effective (Yoder *et al.* 1984), even given a heavy inoculum carryover from the previous season.

Bravo^R 500 (chlorothalonil), one of the Nitrile fungicides, is popular with growers for the control of leaf curl because it also protects against brown rot of stonefruit and can be applied at blossom. Bloom applications have given complete or near complete control of curl (Zehr *et al.* 1983; Burr and Smith 1984). It is less effective than Ferbam^R or Kocide in autumn, autumn and delayed dormant and delayed dormant applications only (Tucker and McGlohon 1984; Drake 1984). Drake found that dormant spring application of Bravo^R 500 provided better control of leaf curl on Redhaven and Newhaven peach and Mericrest nectarine than dormant late autumn applications of Bravo^R, Ferbam^R or Basic copper. Drake

also observed that the foliage of the Bravo^{R} spring treated trees was more dense and vigorous than that of trees treated with Ferbam^{R} or Basic copper fungicide but did not make any objective measurements. Drake suggested that phytotoxicity may have occurred with Ferbam^{R} or basic copper.

Zehr *et al.* (1983) were surprised at their results as sprays applied during bloom were often too late to control *T. deformans*. The effectiveness of such blossom sprays may have been luck in that the infection period must have occurred shortly after or at the end of bloom. The efficacy of all fungicides is thus very dependent on the timing of applicatons relative to the infection period as discussed above.

The Quinone Dichlone controlled leaf curl in mature trees of Blake peaches in South Carolina, USA, but the Pyrimidine Rubigan^{R} did not (Zehr *et al.* 1983).

Not all reports on the effectiveness of fungicides to control peach leaf curl are consistent. For example, Matthee and Kriegler (1975) found that whilst an autumn application of copper oxychloride was more effective against leaf curl than an application at bud swell, the reverse was true for thiram. On the other hand, autumn applications of copper were effective in controlling leaf curl in the following spring in California (Wilson 1937).

Fungicides so far discussed are all protectants rather than eradicants of the leaf curl fungus. In the search for an eradicant fungicide Cheah and Tate (1986) demonstrated complete inhibition of the overwintering stage of *T. deformans* in laboratory tests of some systemic-acting ergosterol biosynthesis inhibitor (EBI) fungicides at concentrations less than 1 mg/kg. In field studies they found that some EBI fungicides were effective against leaf curl when used as protective sprays at bud movement. However, because of the systemic activity of many EBI fungicides they suggested they would be better applied during blossom, when the green leaf first appears. This was tested in 1987 but was not as effective as standard protectants. Thus EBI fungicides were found to have limited use against leaf curl (Tate *et al.* 1989 unpub).

2.4.2.3 Summary

The two lifecycle stages of *T. deformans* susceptible to fungicides are (i) leaf fall, as ascospores produce yeast-like colonies of overwintering spores; and (ii) at the beginning of bud swell in spring, when germinating spores are present. When these periods occur in wet conditions and in temperatures between 10°C and 21°C, infection of *T. deformans* occurs.

To control peach leaf curl fungicides must kill spores, especially those germinating. There must also be

enough chemical present on the buds for rain to wash some of the fungicide in between the bud scales and kill any spores lodged there (Heyns 1965). Once the fungus has entered the leaves no chemical yet discovered will control it, although there has been some laboratory success with the use of EBI systemic fungicides (Tate *et al.* 1989) it was not repeated in field trials.

The standard control for leaf curl has been Bordeaux mixture at bud movement (Atkinson 1968), but before buds opened. More recently, fixed copper fungicides (cupric hydroxide and copper oxychloride) have become popular because they are easier to use and handle than Bordeaux (Tate *et al.* 1985). Whilst some coppers can result in phytotoxicity they are still the preferred fungicide because of their combined effect against other diseases.

Protectant fungicides such as copper oxychloride or captafol^{R} need to coincide with the infection period for leaf curl. Common commercial practice is two spray applications, one as the buds start to move and another a week later. This ensures timing is right and full coverage is obtained. Autumn sprays may not be effective in high winter rainfall areas (Booth 1981).

Replacements for copper fungicides have been and still are actively sought. Some Pthalimides and Dithiocarbamates proved as effective as the copper fungicides, with less phytotoxicity but do not also control bacterial diseases such as stonefruit blast, bacterial canker or gumspot as the coppers do. In New Zealand, where up to five Bordeaux or fixed-copper sprays can be applied between autumn and late dormancy to control bacterial diseases, the additional use of sprays such as captafol^{R} to control peach leaf curl was not justified (Tate *et al.* 1985).

Bravo^{R} 500 (chlorothalonil), one of the nitrile fungicides, is popular with growers for the control of leaf curl because it also protects against brown rot of stonefruit and can be applied at blossom.

2.4.3 Seaweed

The greatest use of seaweeds occurs in Europe, Japan, and North America, principally for fertiliser. Seaweeds were used centuries ago by ancient Greeks, Vikings and the Chinese as soil conditioners, or mulches. In the late 1940s seaweed extracts were developed commercially (Abetz 1985) for use as liquid fertilisers. In New Zealand there is no legal definition of a seaweed extract but they are legally not fertilisers because they contain less than 3% nitrogen, potassium or phosphorus. Notes on commercial New Zealand seaweed extracts are in Appendix D.

Scaweed extracts are concentrates of seaweed which are diluted with water before use, the dilution varying from 1:50 (aerial applications) to 1:1000 (overhead irrigation systems). For most crops the annual application rate is 10 l/ha. A typical algit seaweed contains 60% soluble organic matter and 32.5% insoluble organic matter; 1.9% potassium; 0.87% nitrogen and 0.14% phosphorus (Porteous 1986 pers comm).

Properties of seaweed extracts

Seaweed extracts improved crop quality and enhanced resistance to pest and diseases and marginal frosts (Chase 1984a). Liquid seaweed extracts are not claimed to be fungicides or insecticides. However, some farmers, observed crop pests such as greenfly, whitefly, red spider mite and other mites on their crops, but found the crop damage at an acceptable level after seaweed applications (Chase 1984b). Aitken *et al.* (1965) suggested seaweed improved the resistance of some plant species to insects and diseases.

One manufacturer claimed the foliar application of their liquid seaweed extract assisted plant chlorophyll production through the stimulation of leaf bacteria (Chase 1986) but did not explain further.

Dr Senn demonstrated at Chemson University that seaweed improved respiration in seeds and seedlings, and that "timely" applications promoted growth and yields of plants (Aitken *et al.* 1965). It was suggested seaweeds promote plant growth by providing nutrients and growth hormones such as cytokinins.

Cytokinins and betaines in seaweed extracts

Cytokinins are naturally occuring plant hormones, essential for plant growth and involved in cell division and enlargement, protein synthesis and chlorophyll production. Betaines are modified amino acids closely involved in the plants osmotic processes and produce similar effects to cytokinins. Some seaweed extracts contain glycine betaine which is known to improve frost resistance (Chase 1986).

Sanderson and Jameson (1986) found a complex of cytokinins present in a New Zealand seaweed extract. They also identified cytokinin glucosides and indole-3-acetic acid in freshly made up seaweed extract. The latter contained about 1.3 mg of cytokinins/litre. When diluted to rates used on horticultural crops this concentration was still sufficient for physiological activity. Williams *et al.* (1981) found extraordinarily high levels of cytokinins in this same extract (up to 200 mg kinetin equivalents per litre). The reasons for this dramatic difference are unclear but suggest that a major problem with seaweed extract manufacture could be uniformity of product.

A number of the claimed responses to seaweed extracts are similar to those obtained following application of known plant growth substances, for example, increased fruit set (auxins and gibberellins), reduced fruit drop (auxins) and improved fruit quality (auxins, cytokinins and gibberellins). A New Zealand seaweed extract, Maxicrop, contained cytokinins of potential physiological activity, which, if taken up by the plant, would not immediately degrade to inactive compounds (Sanderson and Jameson 1986). The auxin indoleacetic acid was also identified in the extract.

Cytokinin production by the fungus *T. deformans*

Studies have shown that the peach leaf curl fungus releases both auxins (Crady *et al.* 1959; Trione 1964; Somner 1961) and cytokinins (Johnston *et al.* 1974). It is thought that immobile cytokinins rather than the mobile auxins were responsible for hypertrophy and hyperplasia in peach leaves infected with *T. deformans* (Johnston *et al.* 1974).

Effect of external cytokinin applications

The value of cytokinins in foliar applied seaweed extracts is unknown. Externally applied combinations of auxins and cytokinins by Johnston *et al.* (1974) to peach leaves and buds of growing plants did not produce symptoms of peach leaf curl disease. The exogenously applied growth hormones could not penetrate the bud scales or the cuticles of the leaves and it is unlikely they would cause hypertrophy or hyperplasia themselves. It was speculated therefore that the intercellular fungal hyphae release cytokinins to the surrounding mesophyll cells inside the leaf at a constant rate over a long period (Johnston *et al.* 1974).

Control of *T. deformans*

Peach trees resistant to peach leaf curl after applications of seaweed extracts have been observed but not confirmed with statistics. One anecdotal report of the effectiveness of seaweed extracts was that of a New Zealand home-gardener who claimed total success with concentrated home-made liquid seaweed sprayed at bud burst and two weeks later onto Blackboy peach trees (Castle 1984).

Foliar spray applications of seaweed extracts on peach trees in June (ie early summer) reduced moulds and post-harvest crop losses (Aitken *et al.* 1965) and could be responsible for reduced insect populations on plants. Orchardists who used Seagro, a New Zealand manufactured seaweed extract, reported healthier foliage, better flavoured fruit, higher resistance to pests (aphids, red spider mite) and resistance to frosts (Seagro 1986 brochure). Again, their observations were not confirmed with statistical data.

One New Zealand manufacturer of seaweed extract quoted reports from Floridan growers of a decreased incidence of *Xanthomonas vesicatoria* (bacterial scab of tomatoes), *Xanthomonas campestris* (black rot of crucifers) and *Peronospora cubensis* (downy mildew) following use of the company's seaweed extracts. These results were based on observations and were not part of statistically evaluated experiments. This company established trials in 1986 to determine if their products had any fungicidal properties but results have not been released to date.

It is not known if seaweed extracts control peach leaf curl. Anecdotal reports of the effectiveness of seaweed for the control of peach leaf curl may be observations of the characteristic shedding of the first formed infected leaves and subsequent growth of healthy leaves. Or, in the case of blackboy peaches, be a variety more resistant to leaf curl than others. However, it is possible that seaweed extracts have some fungitoxicity, given the observed decreases in disease incidence reported in the general literature.

Seaweed extract manufacture

The production of commercially available seaweed extracts varies. Most seaweed extracts are made by aqueous or cold extraction processes. Some are heat treated, others are vacuum processed and can have acids, alkalis, enzymes or booster chemicals used or added to convert the dried kelp meal into liquid seaweed. Seaweed extracts made by a low temperature aqueous process prevent the loss of hormones (Chase 1984b).

Seaweed extracts are obtained from different brown seaweeds such as *Ascophyllum nodosum, Fucus* sp. and *Laminaria* species (Chase 1984b). These are common in Gulf stream waters, especially off the coasts of Ireland, Scotland, Norway and Iceland.

2.4.4 Other controls

Burchill *et al.* (1976) obtained effective control of peach leaf curl over two seasons with 2 winter sprays of a 63% mixture of fatty acid alcohols (Off-Shoot T) applied at 5% active ingredient. Although phytotoxicity was insignificant, they thought that leaves emerging from sprayed trees were slightly smaller than those from the control. Trees were too young to fruit so further work is needed to assess the effect on fruit bud formation, fruit set and yield.

2.5 METHODS USED TO EVALUATE THE CONTROL OF PEACH LEAF CURL

2.5.1 Laboratory evaluation

Cheah and Tate (1986) described a rapid technique for the quantitative measurement of the growth of the leaf curl fungus in culture. They tested *T. deformans* isolates for resistance to current and new fungicides.

Spore suspensions of *T. deformans* were prepared from a 14 day old culture. One millilitre of this suspension was seeded onto the agar surface of various culture media in petri plates. The suspension was evenly spread with a glass rod to ensure complete coverage. Excess suspension was then removed with a capillary pipette, leaving a thin film of spores covering the agar surface. Tests were made to show that the spores were evenly spread on the agar surface.

Initial spore concentrations were estimated by taking a 10 mm diamater agar plug from the centre of each plate, vigorously shaking it in a test tube containing 10 ml of water and counting the number of spores present with a haemacytometer. Final spore concentration was assessed using the same procedure four days after incubation at 20°C. Growth of the fungus was calculated as the difference between initial and final spore numbers.

To test for resistance to fungicides a PDA and peptone medium was amended with captafol or copper at four concentrations (500, 1000, 2000 and 3000 mg ai/l). Four isolates of *T. deformans* from different localities were tested and growth assessed with haemacytometer counts. Growth of all isolates was completely inhibited at 500 mg ai/l for captafol and 1000 mg ai/l for cupric hydroxide.

Cheah and Tate found the screening technique a simple and rapid method to quantify the growth of fungi such as *T. deformans* in culture. Advantages of agar plates over broth culture were that growth of the fungus could be both visually and quantitatively assessed within four days compared with 21 days for the PDA broth cultures of Mix (1953) and contaminants could be easily removed without infecting the rest of the culture.

2.5.2 Field evaluation

Most researchers (Tucker *et al.* 1984; Yoder *et al.* 1984; Zehr *et al.* 1983) used a randomized complete block design with replications. The number of replications varied but was dependent on tree age and plant density. Owing to the small number of trees involved in experiments most applications of

fungicide were made by hand spraying trees to run-off.

Disease assessment varied slightly between researchers but generally an estimate was made of the percentage of leaves curled about four weeks after full bloom. When more mature trees were used, for example by Zehr *et al.* (1983), a number of shoots (about 100) were selected at random and examined for peach leaf curl. Commercially control is considered adequate when less than 2% of the shoots are diseased.

Fitzpatrick (1934) applied a series of lime sulphur sprays to separate branches of mature trees throughout the early spring. She graded harvested leaves from randomly selected branches as healthy (no curl), slightly curled (< 25% leaf surface diseased), moderately curled (25-75% leaf surface diseased), and completely curled (>75% leaf surface diseased).

One of the problems of this method is that infected leaves on a branch act as a source of inoculum for other leaves on the tree, especially in rain. It is not clear from Fitzpatrick's paper how many branches per tree were used, nor where the branches were situated on the tree.

The most recent study of fungicides for the control of peach leaf curl in New Zealand was by Tate *et al.* (1987) who evaluated captafol (Difolatan^{R}), four ergosterol biosynthesis inhibitor fungicides (prochloraz, diniconazole, myclobutanil and hexaconazole) and others. They used a randomised complete block design but had only single tree plots. There were four replicates of 3 year old Fantasia nectarines and two replicates of 3 year old Springcrest peaches. Both were at high density planting. All trees were inoculated by spraying with a pure culture of the yeast stage of the fungus (200 000 cells/ml) on 5 August to promote the development of the disease. Trees were sprayed to drenching with fungicide at flower bud movement (4 August), early bloom (15 August) and leaf emergence (28 August). Two methods were used for the assessment: a) 5 one year old laterals were selected per tree and all green shoots classed as either diseased or healthy; and b) terminal shoots on all trees were classified similarly. The proportion of diseased to healthy shoots was calculated.

2.5.2.1 Statistical analysis

Researchers have tended to use transformations of percentage disease data, generally arcsin transformations, to give a linear, additive model and enable ANOVA calculations. Data has then been presented as back-transformed means. The limitations of this presentation are that the back-transformed data have both different means and different variances to the transformed data and what may be a difference in the transformed data may not be true for the back-transformed data. Thus the results can

only correctly refer to the transformed data. In most leaf curl fungicide studies this is the arcsin of percentage disease, which is a difficult concept to discuss with biological data.

A more appropriate analysis is the use of chi-square tests which compare observed data with expected data. This method was not commonly used by researchers.

2.5.2.2 EPPO guidelines

The European and Mediterranean Plant Protection Organisation (EPPO) set guidelines in 1984 for the biological evaluation of fungicides for the control of *T. deformans*. Treatments were to be in a randomised design with a minimum plot size of 3 trees and at least 4 replicate blocks. Records were to include data on daily rainfall and daily maximum and minimum temperatures around the time of spray applications. Throughout the trial records of extreme weather conditions, such as severe or prolonged drought, heavy rain, late frosts, hail etc, which are likely to influence the results were also to be recorded.

A single assessment on 10 marked branches per plot, randomly selected all around the trees and at different heights was sufficient. On each branch curled leaves and uninfected leaves on 10 young shoots from the tips downwards were scored. Alternatively the percentage of leaf clusters on each shoot were to be recorded and the cluster scored for disease intensity on a scale of, for example, 1-5.

EPPO suggested the crop be examined for phytotoxicity and any effects on other organisms noted. No qualitative or quantitative recording of yield was considered necessary.

3.0 LABORATORY EXPERIMENTS

This section describes the laboratory experiments done to observe the leaf curl fungus. Various media were assessed (section 3.2.1), and methods were developed to compare different isolates of *T. deformans* (section 3.3) and later to evaluate the effect of fungicides and seaweed extracts on the growth of the fungus (section 3.4 and 3.5).

3.1 GENERAL METHODOLOGY

3.1.1 Seeding of *T. deformans*

Unless otherwise stated, cell suspensions were made by placing three bacteriological loops of *T. deformans* removed from the surface of PDA plates into 20 ml of distilled water. For petri dish inoculation 0.5 µl of this suspension was seeded onto the media surface and evenly spread with a glass rod. Excess suspension was removed with a capillary pipette, leaving a thin film of cells on the agar surface.

3.1.2 Statistical analysis

Results were analysed for significant differences between the means by analysis of variance using the SAS (Statistical Analysis System) programme, in particular, the General Linear Model procedure. Field results were also analysed using chi squares.

3.2 DEVELOPMENT OF METHODS FOR THE GROWTH OF *T. DEFORMANS IN VITRO.*

3.2.1 Media

Introduction

Initial experiments aimed at finding an appropriate medium for the satisfactory growth of *T. deformans in vitro*. Factors considered in the evaluation of media included the time taken before growth of *T. deformans* was observed, preparation time of the media and the cost of materials. Research (Martin 1940) has shown that *T. deformans* grows well on media of pH 3.5.

Materials and method

An isolate of *T. deformans* from Dr Cheah of Levin Horticultural Research Station (LRC) was used to test media. The following media were prepared in December 1986 (refer Appendix C for media recipes):

1. malt agar and peptone;
2. potato dextrose agar (PDA) and peptone (PDAP);
3. czapek (Cz);
4. acidified PDA (APDA); and
5. fresh potato extract (FPE).

Seeding of media with *T. deformans*

A suspension of 5.27 x 10^6 cells/ml of *T. deformans* was made by adding 5 bacteriological loops of fungal culture growing on PDA to 20 ml of sterile distilled water. One ml of the fungal suspension was added to 400 ml water for the stock inoculant from which 10 µl (1.3 cells average) was used to seed petri plates of each medium. After seeding, the plates were incubated at 20°C for four days. Growth of *T. deformans* was observed and cells from the centrifuged and non-centrifuged plates were counted, using a haemacytometer.

Results

T. deformans grew well on both PDAP and FPE, more slowly on malt agar and peptone and not at all on Cz. Table 3.1 shows the number of cells found in the control and centrifuged fresh potato extract media. Centrifuging made little difference to the visibility of cells under the microscope and no difference to

cell counts. *T. deformans* growth on APDA was the same as on non-acidified PDA.

TABLE 3.1

GROWTH OF *TAPHRINA* AFTER 4 DAYS ON FRESH POTATO EXTRACT MEDIA

Extract	Centrifuged (no./ml) *	Not centrifuged (no./ml) *
Potato only	75.4×10^6	95.5×10^6
Potato, dextrose (12g/l)	30.5×10^6	22.5×10^6
Potato, dextrose, lactic acid (1 ml/l)	8.2×10^6	11.3×10^6

*Initial suspension: 5.27×10^6 cells/ml

Discussion

T. deformans grew adequately on fresh potato extract and on commercially prepared PDA. Centrifuging FPE was unnecessary as cells were as visible (at x400 magnification) on non-centrifuged slides. For speed, economy and efficiency of later experiments commercially prepared PDA and 1% peptone were used for growth of *T. deformans in vitro*. No adjustment was made to pH.

3.3 COMPARISON OF DIFFERENT ISOLATES OF *T. DEFORMANS*

Introduction

Two isolates of *T. deformans* were obtained from infected leaves of local trees but attempts made to infect peach shoots in the laboratory to prove pathogenicity were unsuccessful. Consequently, these two isolates were used in initial work only. Later work used a named isolate from Dr Cheah of Levin Research Centre and another isolate from the International Collection of Micro-organisms From Plants (ICMP) in Auckland.

3.3.1 Isolation of *T. deformans*

Materials and method

Two methods were used to isolate *Taphrina deformans*:

a) In late November, 1986, peach leaves showing symptoms of peach leaf curl were surface sterilised in a 6.3 g/l solution of NaOCl for 2 minutes. They were rinsed in sterile distilled water and 0.5 cm diameter leaf sections placed onto potato dextrose agar media (PDA) amended with 1% penicillin and 1% streptomycin. After three days incubation at 20°C the fungus growing out of the sections was transferred to fresh PDA plates for further incubation. The isolate obtained from this method was labelled **A**.

b) The second method was similar to the above but leaves were rinsed in sterile water, not surface sterilised. Sections of the leaves were placed on wet filter paper on an inverted PDA plate and the antibiotic amended PDA placed above them. The disks were incubated for 5 days at 20°C. Ascospores ejected onto the agar were removed with a sterile needle tip and plated onto selected media. An isolate from this technique was called **B**.

Oil immersion slides were prepared by combining the fungus with oil and smearing it onto slides. Four and 17 day old cultures were studied under x400 magnification.

Results and discussion

Isolate A grew faster than B and was more of a cream colour than the pale pink of Isolate B. Cells of both isolates were about 5 µm. There were more cells budding in the old culture of A than in the old culture of isolate B. There were many thick walled round cells and some seemingly budding cells in the old isolate of B. They were about 2.5-5 µm. No such cells were seen in the young sub-cultures.

Isolate B ressembled Kramer's (1973) and Fitzpatrick's (1934) description of *T. deformans* more than did A (refer section 2.3.3).

3.3.2 Confirmation of the isolates as *T. deformans*

To confirm the two isolates A and B as *T. deformans* an attempt was made to induce infections of *T. deformans* on peach cuttings, taken in summer. The technique of growing peach cuttings in the laboratory over summer was not successful and confirmation of the pathogenicity of the isolates was not possible. Problems incurred in using this method are described.

Materials and method

Tip cuttings, about 25 cm long, of Flamecrest peach were taken in the first week of January, 1987. These were stripped of leaves and surface sterilised in 6.3 g/l of NaOCl for two minutes. They were placed in jars of water, covered loosely with individual plastic bags and left in an incubator at 20°C for 4 days.

Cell suspensions of isolates A and B were prepared by placing 5 loopfuls of each isolate into 20 ml of sterile distilled water in McCartney bottles. One ml of each suspension was put into 2 jars, each containing 200 ml of fresh potato extract medium (Appendix C). After 2 days incubation at 20°C the cells in both cultures were budding. Suspensions of A,B and a control of water were then painted onto leaf buds of the peach cuttings. The cuttings were covered in plastic and kept at 20°C.

The petri dish zonal inhibition test (described in section 3.3.1) was used to compare the response of different isolates to copper oxychloride.

Results and discussion

After 2 days, the plastic covering the cuttings was removed because the cuttings were overheating. Without the plastic, the cuttings dehydrated because the humidity could not be regulated.

The experiment was repeated at the end of January, 1987 and cuttings were placed in a humid chamber for five days. Again there was no success in initiating growth of any leaves and inoculation with the isolates was not possible.

It was beyond the scope of this study to establish better procedures for the growth of peach seedlings in the laboratory so the work of other researchers (Martin 1940, Mix 1935) was used as a basis for

information on the growth and morphology of *T. deformans*.

Since the confirmation of the isolates A and B as *T. deformans* was not possible two isolates were obtained from other sources for further work:-

1. LRC isolate: from Dr Cheah of MAFTech Levin Horticultural Research Centre;
2. DSIR isolate: a named culture *T. deformans* (Berkeley) L.R. Tulase 2924, from the International Culture Collection of micro-organisms at Plant Diseases Division, Department of Scientific and Industrial Research, Mt Albert, Auckland. It was isolated in New Zealand from a peacherine, *Prunus persica* (L.) Batsch and had been stored freeze dried.

Comparison of isolate B, the LRC isolate and the DSIR isolate was made using the petri dish zonal inhibition test, described in section 3.3.1.. All isolates were inhibited at 1000 ppm of copper oxychloride.

The DSIR and LRC isolates were significantly different to each other in both their growth on PDA and peptone media and in their reaction to copper (refer Table 3.3). The DSIR isolate had a different overall appearance: it was a brighter pink and more vigorous in growth than the LRC sample. The inhibition zone of the DSIR isolate at 100 and 500 ppm of copper oxychloride was unusual and suggested either the inadequacy of this method for the testing of fungicide inhibition or an unusual strain of the fungus. Later confirmation of the DSIR isolate as a yeast and not *T. deformans* explained its unusual behaviour in these experiments.

Label rates for peach leaf curl control with copper oxychloride are 2500 ppm of active ingredient/l at bud-burst and 1500 ppm 10 days later. In testing for resistance of *T. deformans* to copper Tate and Cheah (1986) found complete inhibition of *T. deformans* at 1000 ppm (and higher) of cupric hydroxide and concluded that the isolate was not resistant to copper since inhibition occurred at concentrations well below the recommended field rates. The inhibition zone experiment (section 3.4.1) confirmed the inhibition of the LRC isolate to copper oxychloride at 1000 ppm.

3.4 DEVELOPMENT OF METHODS TO EVALUATE FUNGICIDES FOR CONTROL OF *T. DEFORMANS* IN CULTURE

3.4.1 Petri dish zonal inhibition technique

Introduction

The petri dish zonal inhibition technique was evaluated by comparing growth of three *T. deformans* isolates (LRC, DSIR and B) at different concentrations of copper oxychloride.

Materials and method

In early March, 1987, five filter paper disks were soaked for a few minutes in a copper oxychloride concentration of either 0; 10; 100; 500; or 1000 ppm. Excess solution was removed by tapping each disk twice against the inside wall of the glass tube. The five disks were laid equidistant on the agar surface of a *T. deformans* seeded petri plate and incubated for three days at 20°C. Presence or absence of inhibition zones was observed.

Results

Table 3.2 shows inhibition zones observed after 3 days. All isolates had some inhibition at 1000 ppm of copper oxychloride and the DSIR and B isolates were also inhibited at 500 ppm. The DSIR isolate also showed inhibiton at 100 ppm. The recommended field rate for copper oxychloride is 1250 ppm.

TABLE 3.2

OBSERVATIONS OF INHIBITION ZONES OF *T. DEFORMANS* TO COPPER OXYCHLORIDE

Isolate	fungicide concentration (ppm) where inhibition zone observed
1. B	500, 1000 ppm
2. DSIR	100, 500, 1000 ppm
3. LRC	1000 ppm

The inhibition zone technique may be unsatisfactory for fungicide evaluation given the unusual response of the DSIR isolate (found to be a yeast). However, the inhibition observed at 1000 ppm in the LRC and B isolates was expected so it was decided to evaluate this, and other, methods for quantification of the effect of fungicide-amended media on *T. deformans* growth.

3.4.2 Quantification of *T. deformans* growth on fungicide-amended PDA

Introduction

The inhibition zone experiment showed that growth of *T. deformans* was inhibited by copper oxychloride at 1000 ppm. The following experiment was an attempt to quantify that effect by use of haemacytometer counts of cells on fungicide-amended media. A further comparison of the LRC and DSIR isolates was made also.

Materials and method

Three replicate plates of 0, 100, 500 and 1000 ppm copper oxychloride amended PDA were poured and *T. deformans* isolates seeded onto them. After incubation for four days at 20°C, three 10 mm plugs were taken and placed in 10 ml of water. A few drops of formalin were added to stop further sporulation. Initial and final cell concentrations were estimated by using a haemacytometer.

To estimate the number of readings per sample, six counts of cells were made from one control bottle.

Results

Table 3.3 shows the growth in cell number after four days on fungicide-amended PDA. The LRC isolate was sensitive to copper at concentrations of 500 and 1000 ppm. A significant difference from the control was also observed in the DSIR isolate but cell numbers were still very high at all concentrations. The DSIR isolate had significantly more cell numbers than the LRC isolate, on all media.

TABLE 3.3

GROWTH OF *T. DEFORMANS* AFTER 4 DAYS ON COPPER-AMENDED PDA

Fungicide concentration (ppm)[1]	Growth (no. cells per 10 mm plug) LRC isolate	DSIR isolate
0	1281 A	6216 A
100	1004 A	5778 A
500	-209 B	3467 C
1000[2]	-391 B	4654 B

Means within a column with the same letter are not significantly different at the 5% level.

1. Cell no. x 2 000 = cells/ml.
2. Recommended field rate to control peach leaf curl = 1250 ppm.

Discussion

Cell counts given in Table 3.3 confirm the observations of the inhibition zone experiment described in 3.4.1. *T. deformans*, as the LRC isolate, was inhibited at concentrations of copper oxychloride lower than the recommended field rate of 1250 ppm. Significant inhibition occurred at both 500 and 1000 ppm.

Since the DSIR isolate was confirmed later as a yeast and has been discussed fully in section 3.3.2, no further discussion of it is made here.

The technique used in this experiment enabled quantification of the effect of fungicides on the growth of *T. deformans*. Further refinement of the method was needed to improve efficiency and reduce costs and is discussed in the next section.

3.4.3 Development of a more economic and efficient method

Introduction

In previous experiments *T. deformans* was grown on PDA in petri plates and growth was measured by counting cells found in a measured plug of PDA in a known quantity of water. An even seed layer was necessary for this method and was not always obtained. Thus plug samples may not have had even distributions of the cell populations. In this experiment small Bijou jars were used instead of petri plates so that all cells grown could be counted.

Materials and method

Two ml of molten PDA was dispensed into Bijou jars (22.5 mm in diameter). *T. deformans* was seeded into jars at volumes of 5, 10, and 15 µl. There were 3 replicates of each volume. After 7 days, 10 ml of distilled water was pipetted into the jars. Jars were shaken vigorously and cell numbers counted.

Results and discussion

Table 3.4 shows the haemacytometer counts of cells from the different seed volumes.

TABLE 3.4

COUNTS AT DIFFERENT SEED VOLUMES

	Number of cells [1]							
SEED (µl)	REP 1		REP 2		REP 3		MEAN	SEx
5	82	52	60	75	60	74	67	12
10	95	70	72	86	86	89	83	10
15	114	143	118	123	118	147	127	14

1. cell no. x 2500 = no. cells/ml

For Bijou jars, 15 µl of seed adequately covered the media surface and provided sufficient seed for cell counts. Growth of *T. deformans* from seed of 5 and 10 µl barely covered the agar surface after 7 days and counts were much lower than those of the 15 µl.

Bijou jars were an economic and effective alternative to petri plates for the evaluation *T. deformans* growth.

3.5 EVALUATION OF FUNGICIDES AND SEAWEED EXTRACTS FOR THE CONTROL OF *T. DEFORMANS IN VITRO*

3.5.1 Evaluation of seaweed extracts and fungicides for control of *T. deformans.*

Introduction

To compare the growth of *T. deformans* on fungicide and seaweed-amended media the method described in section 3.4.2 was used. Materials tested in this experiment included two fungicides, copper oxychloride and captafol^{R}, and seaweed extracts. The Ecklonia and Carpophyllum extracts both contained a preservative but the Pterocladia extract did not. The base liquid for Response is a combination of Ecklonia and Pterocladia to which chelates and applicable N P K are added. The Seagro extract is made from Algit and incorporates a similar preservative. Nipasol-M-Sodium is the preservative used in another common liquid seaweed extract and was tested for fungicidal properties in this experiment. Further information on seaweed extracts is given in appendix D.

Materials and method

Autoclaved PDA solution was dispensed in 40 ml amounts into 19 x 100 ml flasks. Flasks were re-capped and placed in a 50°C water bath. Treatment solutions, made up separately in McCartney bottles, were added to the flasks of PDA to give a total volume of 50 ml. The treatments were not autoclaved. Two ml of the resultant mixture was dispensed into 3 small jars, labelled and left to set. The remaining media was poured into petri plates.

A suspension of 4.55×10^6 cells/ml of *T. deformans* was made from 29 day old LRC isolate. Jars were seeded and placed at 20°C. Each jar contained about 68 250 cells (0.015 ml x 4.55×10^6 cells/ml).

Observations were made after two days. After six days five mls of distilled water was added to each jar and cells were shaken into suspension. One ml of this suspension was placed in another five mls of distilled water and cell numbers were counted using a haemacytometer. Final concentrations were adjusted for dilutions to give the correct number of cells. Three counts were made of each replicate of each treatment. Results were analysed using the general linear model procedure of the SAS computer programme to compare differences between means.

Treatments were as follows:

	Concentration
1. Control	10 ml sterile distilled water
2. Copper oxychloride	1000 ppm (0.05g a.i./50 ml)
3. Copper oxychloride	4000 ppm (0.2g a.i./50 ml)
4. Captafol	250 mg a.i./l

5.	Captafol	500 mg a.i./l
6.	Captafol	800 mg a.i./l (recommended field rate)

Seaweed extracts:

7.	Seagro	10 ml/l
8.	Seagro	20 ml/l
9.	Response	3.3 ml/l
10.	Response	100 ml/l
11.	Nipasol-M-Sodium	10 ml/l
12.	Nipasol-M-Sodium	1 ml/l
13.	Nipasol-M-Sodium	0.1 ml/l
14.	Ecklonia	100 ml/l
15.	Ecklonia	10 ml/l
16.	Carpophyllum	100 ml/l
17.	Carpophyllum	10 ml/l
18.	Pterocladia	100 ml/l
19.	Pterocladia	10 ml/l

Results

TABLE 3.5
***T. DEFORMANS* GROWTH ON FUNGICIDE AND SEAWEED AMENDED MEDIA**

Treatment		Mean no. spores after 6 days ($\times 10^6$)
1.	Control	235.b[1]
2.	Copper oxychloride 1000 ppm	0.d
3.	Copper oxychloride 4000 ppm	0.d
4.	Captafol^R 250 mg a.i./l	0.d
5.	Captafol^R 500 mg a.i./l	0.d
6.	Captafol^R 800 mg a.i./l	0.d

Seaweed extracts:

7.	Seagro 10 ml/l	13.5a
8.	Seagro 20 ml/l	1186.c
9.	Response 3.3 ml/l	207.b
10.	Response 100 ml/l	*.
11.	Nipasol-M-Sodium 10 ml/l	154.b
12.	Nipasol-M-Sodium 1 ml/l	127.b
13.	Nipasol-M-Sodium 0.1 ml/l	209.b
14.	Ecklonia 100 ml/l	*.
15.	Ecklonia 10 ml/l	303.b
16.	Carpophyllum 100 ml/l	*.
17.	Carpophyllum 10 ml/l	166.b
18.	Pterocladia 100 ml/l	*.
19.	Pterocladia 10 ml/l	*.

[1] Means with the same letter are not significantly different at 5%

* *Taphrina* cells could not be distinguished from bacteria and other fungi contamination.

Slight growth was observed after two days in one of the controls. After six days growth of *T. deformans* was observed in all the controls, both Response treatments (9 and 10), the Nipasol treatments, the 10 ml/l Ecklonia treatment and the 10 ml/l Carpophyllum treatment.

Contamination occurred in the higher concentrations (100 ml/l) of the extracts Response, Ecklonia and Carpophyllum and prevented the evaluation of *Taphrina* cells. In repeated tests using these extracts, plates again became too contaminated. It is thought the seaweed extracts contained a significant amount of bacteria and fungi themselves and would therefore require sterilising before using for this experiment. These extracts were not evaluated further.

The Seagro extract had a slight effect on the growth of *Taphrina* at 10 ml/l but the result at 20 ml/l negates this. However, it is possible that not all the cells counted in the 20 ml/l treatment were *T. deformans* as there was variability in their outlines (many had a darker outline than the control) and counting was made difficult by cells being clumped together.

Discussion and conclusions

The growth of *Taphrina* cells in the control jars and the lack of growth on the copper and captafol^{R}-amended PDA indicated this was an effective method for assessing fungitoxicity.

There was no evidence of fungitoxicity from the seaweed extracts Response, Pterocladia, Carpophyllum and Ecklonia nor the Nipasol preservative. However, the results of the Seagro extract were inconclusive and warranted further study.

Contamination with fungi and bacteria was a problem with some of the seaweed extracts, particularly the Pterocladia where all plates were too contaminated to differentiate the *Taphrina* cells. Contamination was principally the result of not sterilising the media initially. This was not done as it was thought that if any beneficial organisms were in the extracts they could be destroyed also. The Pterocladia extract was not re-tested as it was a base of the Response extract and it was thought any fungitoxicity would be shown there.

3.5.2 Effect of sterilising seaweed extracts

Introduction

Contamination of plates and jars was a problem in some treatments of the experiment described in 3.5.1. In the experiment described below, an attempt was made to sterilise the seaweed extracts before use to decrease plate contamination with bacteria and other fungi from the extract.

Materials and method

Extracts were too thick to pass through a millipore filter for sterilisation so the necessary volume of each concentrate was put into a sterile flask and 100 ml of autoclaved PDA media was added. Lactic acid was added to bring the pH to 4. Two ml of the resultant solution was dispensed into each of 3 replicate jars and the remainder was poured into petri plates. Twenty µl of fresh cell suspension was dispensed into jars. Plates were streaked with *T. deformans*.

Treatments

Seaweed extract	concentrations (ml/l)	pH of concentrate
Seagro	2.5; 3.3; 5; 10	5.2
Response	5; 10; 20; 40	6.5
Ecklonia	13.3; 20; 40	8.4
Carpophyllum	13.3; 20; 40	8.0
Control		

Results and discussion

T. deformans grew very well at all concentrations of Response, Ecklonia and Carpophyllum and it was concluded that these extracts had no inhibitory effect on the fungus. No further testing of these products was thus made.

Growth was slower on the Seagro plates and it was also observed that there was less growth on the 10 ml/l plates than the lower concentrations.

Contamination of the control plates of *T. deformans* made it impossible to see any growth of the fungus and thus statistical comparison between the control and other treatments was not made. The control plates were poured from the stock solution last and contamination may have occurred towards the end of the procedure.

No counts were made because of the control contamination but the results again suggested that the Seagro extract warranted more study.

3.5.3 Evaluation of fungus isolated from Seagro extract

Introduction

Circular colonies of a white fungus developed on some plates of PDA and Seagro extract. The fungus was isolated into pure culture and tested for antagonism to the peach leaf curl fungus.

Materials and method

Plugs of the Seagro fungus were placed on PDA plates which were then seeded with a *T. deformans* suspension. They were incubated at 15°C for 7 days.

Results and discussion

After 7 days, the unidentified Seagro fungus was found in all but the control plates. It had not affected the growth of *T. deformans* so no further work was carried out and no identification made.

3.5.4 Evaluation of the fungicides Saprol^R, Benlate^R and Rovral^R, and the bactericide Streptomycin on peach leaf curl.

Introduction

Peach trees in the field trial were infected with bacterial blast. The commonly used pesticide for control of blast (Agrimycin) and those commonly used to control brown rot of fruit (Saprol^R, Benlate^R and Rovral^R) were tested for fungitoxicity to *T. deformans*.

Materials and method

Fourteen flasks containing 80 ml of water and the appropriate quantity of PDA were autoclaved. Pesticide concentrations were measured and dissolved in 20 ml of sterile distilled water and added to the PDA solution. Five petri plates of each concentration were poured. *T. deformans* suspension was seeded onto the plates. After 4 days, 10 ml of sterile distilled water was added to the plates and the surface lightly rubbed with a glass rod to ensure all cells were in suspension. Both LRC and DSIR isolates were used in the experiment to further evaluate their differences.

In contrast to other experiments where the number of cells was counted using a haemacytometer, the resultant cell growth was measured using light absorbance in a spectrophotometer.

Results

The two isolates were significantly different to each other in their response to the pesticides. Table 3.5 shows the light absorbance readings of treatments after 4 days growth. Low readings (0-0.2) indicate no

or very little growth of the fungus and high readings indicate the *T. deformans* fungus was able to grow on the media.

TABLE 3.6

SPECTROPHOTOMETER READINGS AT 640NM OF *T. DEFORMANS* AFTER 4 DAYS GROWTH ON PDA AMENDED WITH PESTICIDE

Pesticide	Concentration	Isolate	
		LRC	DSIR
SAPROL^R	(ml/l)		
	0.5	0.56 D	0.65 F
	1.0*	0.01 E	0.06 G
	2.0	0.00 E	0.00 G
BENLATE^R	(g/l)		
	0.2	2.32 ABC	0.99 EF
	0.4*	2.42 AB	1.41 CD
	0.8	2.50 AB	1.05 DE
ROVRAL^R	(g/l)		
	0.375	2.40 AB	1.53 BC
	0.75*	1.98 BC	1.45 C
	1.5	1.85 C	1.60 BC
STREPTOMYCIN	(g/l)		
	0.3	2.55 A	1.84 B
	0.6*	2.40 AB	1.64 BC
	0.9	2.68 A	2.35 A
CONTROL		2.73 A	1.46 C

* recommended field rate
Means with the same letter within a column are not significantly different at the 5% level.

Saprol had a significant inhibitory effect on the growth of the LRC isolate of *T. deformans* but both Benlate^R and Streptomycin, at all rates, were no different to the control. Rovral^R at the lowest concentration (0.375 g/l) was not significantly different to the control but at the recommended field rate (0.75 g/l) and higher it had a significant inhibitory effect on *T. deformans* growth *in vitro*.

The DSIR isolate, later confirmed as a yeast, was significantly different in growth and habit to the LRC. Saprol had an inhibitory effect on the DSIR isolate but Benlate^R, Rovral^R, and Streptomycin, at recommended rates, were ineffective.

Discussion

The spraying of streptomycin to control bacterial blast in the field trial plots should not affect the growth

of *T. deformans*. If a spray to control brown rot in the experimental trees was needed, Benlate^{R} was the best option as it had no significant effect on the growth of *T. deformans*.

4.0 FIELD EXPERIMENT

Introduction

This experiment evaluated the effect of a seaweed extract and resistant varieties of peach for the control of peach leaf curl.

Materials and method

Nectarines (cv. Red Diamond) and two varieties of peach (cv. Gordon's Glory and cv. Winiata Gold), were planted in June 1987 in a a randomised complete block design. There were four replicate blocks. Rows were 4.5 m apart, plots within each row contained three trees 1.5 m apart, with three metres between plots. No guard rows were used because of the distance between rows.

There were seven treatments:

TREATMENT	CONCENTRATION	DATES APPLIED
1. Control	no leaf curl sprays	
2. Seagro (5 sprays @ 3 weekly intervals)	10 ml/l (1:100)	12/8/87 22/1/88 - 7/4/88
3. Seagro	10 ml/l	12/8/87
4. Seagro	5.5 ml/l (1:300)	12/8/87
5. Copper oxychloride	2g/l	12/8/87 4/7/88 28/8/88
6. Winiata Gold	resistant peach	
7. Gordon's Glory	resistant peach	

Sprays were applied to run-off using a low pressure knapsack sprayer. The bud stage at time of spray application is shown in Plate 2. Trees were planted in July 1987 and were too young to produce significant fruit yields during the experimental period. It was not necessary to spray for prevention of fruit diseases such as brown rot.

One streptomycin spray was applied to all trees in mid-October (full leaf) 1987, to control bacterial blast. This was later than the recommended application times of 10-14 days and 28 days after green tip.

A diary of field trial management is given in Appendix B.

Assessment method

Five laterals were randomly selected from each tree and all leaf clusters on the laterals classed as either

diseased or healthy. Assessments were made on the 17 October in both 1987 and 1988. Leaf clusters were classed as diseased if any part of the cluster showed leaf curl symptoms. In 1987 an additional assessment was made as a visual observation of disease incidence of overall tree health on the 25th September (leaflets about 5 cm long). No visual assessments were made in 1988.

Percentages of disease were calculated for each plot and treatments compared using standard chi square tests. Back-transformed data was also used and statistically tested for differences between the means.

Results

1987

Observations of leaf curl incidence were made on 25 September, 1987, nine weeks after bud swell in the Red Diamond nectarines. Leaves were 5 cm on average.

Observation of leaf curl incidence on 25/9/87

	Range of observed % disease per plot
1. Untreated Control	30-50
2. Seagro 10 ml/l (5 sprays)	5-50
3. Seagro 10 ml/l	10-30
4. Seagro 5.5 ml/l	5-50
5. Copper oxychloride	5-10
6. Winiata Gold	10-20
7. Gordon's Glory	0-20

The resistant varieties had later emerging leaves and were overall less infected with leaf curl at this stage. The copper treated trees also had less infection than other treatments. A large number of trees from all treatments were affected with bacterial blast.

Table 4.1 shows the copper oxychloride treatment had the lowest level of disease in 1987 and was significantly different to the untreated control and all Seagro treatments. The two resistant varieties had significantly less disease than the untreated trees and those treated with the higher rate of Seagro. The lowest rate of Seagro had the least disease of the Seagro treatments although the difference was significant in treatment 3 only.

TABLE 4.1

Disease Incidence 17 October 1987 and 1988

TREATMENT	Mean of arcsin transformed data		
	1987	1988	
1. Untreated Control	.385 A B		E
	(35)	(100)	
2. Seagro 10 ml/l	.388 A B		E
(5 sprays)	(36)	(100)	
3. Seagro 10 ml/l	.466 A		E
	(41)	(100)	
4. Seagro 5.5 ml/l	.352 B C		E
	(32)	(100)	
5. Copper oxychloride	.211 D		E
	(20)	(100)	
6. Winiata Gold	.300 C D F	.241	F
	(27)	(25)	
7. Gordon's Glory	.266 C D F	.275	F
	(26)	(26)	

Means with the same letter are not significantly different at the P=0.05 level.

Numbers in brackets refer to the raw percentage disease data.

Analysis of data using chi square (hiloglinear)

1987 data

Treatment	Transformed data	
1. Copper	2.91	LEAST DISEASE
2. Gordon's Glory	3.54	
3. Winiata Gold	3.83	
4. Seagro (5.5 ml/l)	4.72	
5. Control	4.95	
6. Seagro (10 ml/l x5)	5.08	
7. Seagro (10 ml/l)	5.88	MOST DISEASE

Chi square analysis using hiloglinear enabled treatments to be ranked, as shown above. In 1987 the copper treatments and the resistant varieties had significantly less (at P=0.05) disease than the control or the seaweed extract. This analysis confirms the analysis of the back-transformed means shown in Table 4.1.

1988

All treatments, except for the resistant varieties, had complete infection of leaf curl. Aphids and bacterial blast were also problems. The severe infection induced premature defoliation of most trees in October. However, the new leaves were generally clear of infection. The resistant varieties were not

significantly different from each other. Plate 3 illustrates the degree of leaf curl infection in the control plots of Red Diamond nectarines.

Comparison between 1987-1988

Only the resistant varieties were compared between years because complete infection of leaf curl occurred in all other treatments in the 1988.

The two resistant varieties, cv. Winiata Gold and cv. Gordon's Glory had no significant difference in leaf curl infection between the two seasons nor between varieties in arcsin transformed data. In both years they were significantly different from the control trees and the Seagro treated trees. In 1988, when infection pressure was severe, they had significantly less disease than the copper treatment.

Infection Periods Identified from Climate Data

Figures B and C show the main rain periods, temperatures and plant growth stages for July-September (inclusive) in 1987 and 1988. Data was taken from the Palmerston North DSIR Grasslands Climate Station and details of the data are given in Appendix E.

Figure B identifies five infection periods (A-E) for 1987. Period A, July 22 (12 mm rain at a maximum temperature of 12°C) occurred 5 days before bud swell (July 27) and period B, August 4-5, (12 mm rain at a maximum temperature range of 15-19°C) at very early bloom or pink. This was followed by cooler temperatures (12-15°C) for 12 days.

Three late infection periods (C-E) occurred:August 22 (8 mm rain, 16°C); September 2-3 (13 mm rain, 14-16°C); and September 11-13 (33 mm rain 13-18°C. Note that the 18°C was followed by 10 days of maximum temperatures less than 16°C).

Leaf curl was visible on cv. Red Diamond nectarines on the 25 September when leaves were an average length of 5 cm. This was 12 days after the last infection period.

Seagro and copper sprays were applied on August 12, after the first two infection periods.

Figure B

MAIN RAIN PERIODS, TEMPERATURES, PLANT GROWTH, JULY–SEPT. 1987

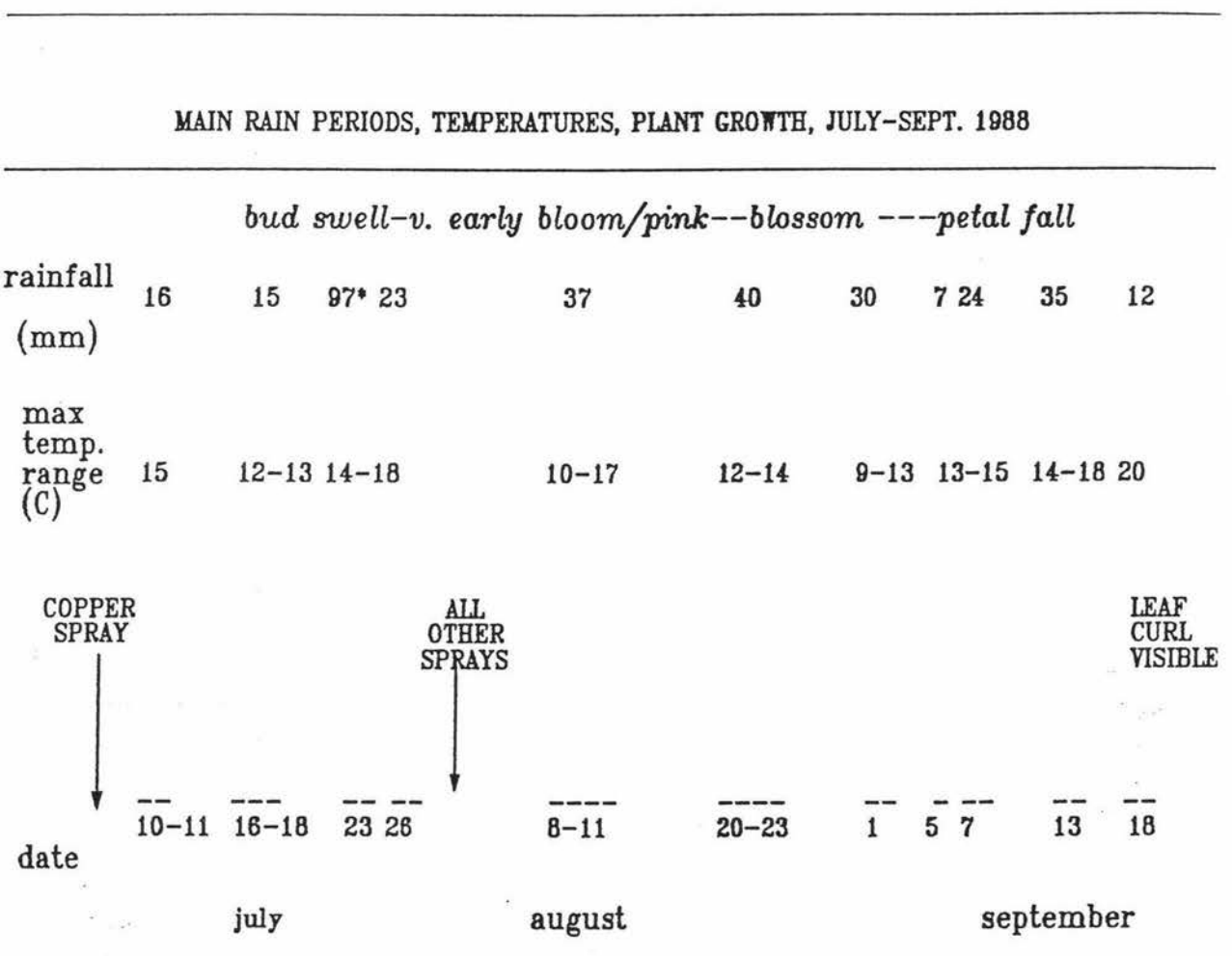
Red Diamond	*bud swell--v. early bloom---blossom ---petal fall*					
Resistant vars.			*pink*			
rainfall (mm)	12	12		8	13	33
max temp. range (C)	12	15–19		16	14–16	13–18
			sprays applied			LEAF CURL VISIBLE
INFECTION PERIOD	A	B		C	D	E
date	– 22	-- 4–5	12	– 22	-- 2–3	--- 11–13
	july		august			september

CLIMATE DATA: GRASSLANDS, DSIR, PALMERSTON NORTH

1988

Figure C shows at least eleven leaf curl infection periods (A-K) for 1988. It was difficult to separate the infection periods as rain was exceptionally high in July (one day had 84 mm) and all of spring. Temperatures during these periods were cool, rarely above a daily maximum of 15°C.

Figure C



FLOOD 82 MM JULY 24

CLIMATE DATA: GRASSLANDS, DSIR, PALMERSTON NORTH

Table 4.2 shows the total monthly rainfall and the average maximum daily temperatures for 1987/1988 compared with the 40 year average. Temperatures were similar for 1987 and 1988, though August was warmer in 1987 than in 1988. Both years were also warmer than the 40 year average for these months.

TABLE 4.2

CLIMATE DATA SUMMARY

	1987		1988		40 year	
Month	total rainfall (mm)	average max. temp. (°C)	total rainfall (mm)	average temp. (°C)	average rainfall (mm)	average max. temp. (°C)
July	33	12.5	163	13.2	91	11.8
August	32	15.1	94	13.8	84	13.0
September	67	15.2	144	15.6	69	14.7
October	93	17.4	98	17.1	89	16.6

(source: Grasslands, DSIR, Palmerston North; 40 yr average 1930-70.)

July and August of 1987 were significantly drier than 1988 and the 40 year average and this, coupled with the warmer temperatures in these months, suggests there were fewer infection periods for leaf curl in 1987 than 1988.

The spring of 1988 was exceptionally wet from July to September (inclusive) and coupled with the cool spring temperatures created ideal leaf curl infection periods. This is one explanation for the extremely high level of leaf curl disease found in the 1988 field trial, even after copper applications.

Discussion and conclusions

The varieties cv. Winiata Gold and cv. Gordon's Glory showed exceptional tolerance to leaf curl, even in the 1988 season when infection pressure was high. The level of tolerance can be seen in Plates 4 and 5 of the resistant varieties. These can be compared with the control plots shown in Plate 3. These varieties need to be tested for fruit yield and quality, and susceptibility to other pest and disease tolerance before recommending to commercial orchardists. Winiata Gold and Gordon's Glory would be useful as a source of tolerance to leaf curl in breeding programmes.

The late application (August) of the copper spray in the 1988 season was because wet and windy conditions prevented spraying any earlier. Spray times compared with Himatangi orchards (30 km SE) for the early and late July applications and the Himatangi orchard was also severely affected with leaf

curl in 1988. The lack of control of leaf curl by the copper fungicide is illustrated in Plates 7 and 8, and compared with the disease incidence in the control plots and the resistant varieties.

The seaweed extract Seagro had no effect on peach leaf curl in the field trial, under a high level of disease pressure. It is possible that in years of low disease pressure it may provide nutrients to trees and thus increase tree vigour and resistance to disease.

The peach leaf curl fungus can penetrate leaves readily at temperatures of 13-18°C (Fitzpatrick 1935). However, it is only leaves infected at the lower temperatures that develop disease. In general, leaf curl is most severe in years when cool, wet weather occurs during bud swell, such as in the 1988 spring. In New Zealand Tate *et al.* (1985), found that the first symptoms of leaf curl were visible on 2 October, 16 days after wet, cool weather and 59 days after leaf bud movement.

The mid-October application of Streptomycin in 1987 did not control bacterial blast and had no effect on the leaf curl assessment for this experiment.

DISCUSSION AND CONCLUSIONS

Leaf curl, caused by the fungus *Taphrina deformans*, is one of several diseases that can severely affect the profitability of peach and nectarine growing. It is potentially damaging to both crop yields and tree longevity , but has in the past been cheaply and effectively controlled.

Tate *et al.* (1987) found that in the last four seasons in New Zealand growers have been very concerned at the increasing difficulty in maintaining effective control of leaf curl. Some applied several additional captafol^{R} or chlorothalonil (Bravo^{R}) sprays, with unsatisfactory results. A strain of *T.deformans* from Hawkes Bay was found in laboratory tests resistant to copper fungicides by Dr Cheah and Dr Tate (Anon 1989) but has to be field tested.

As an exporter of fresh horticultural produce such as nectarines and peaches, New Zealand growers are vulnerable to importing countries restrictions on pesticide residues. These restrictions are dynamic; what may be acceptable at the start of the growing season may not be allowed at the end of it. During the three years of this study the fungicide Captafol^{R} (a.i. difolatan), which was recommended by MAFTech for the control of leaf curl in 1987, was banned for use on stonefruit crops. It is thus necessary to find methods of pest and disease control which avoid the use of potentially controversial chemicals. The need for alternatives to the present fungicides used for the control of diseases such as leaf curl is on-going.

This study found that two varieties of peach, Winiata Gold, and Gordon's Glory, were tolerant to peach leaf curl under conditions of high levels of infection pressure. Growers wanting to reduce their use of copper and other fungicide sprays are recommended to plant varieties resistant to peach leaf curl. The peach varieties Winiata Gold and Gordon's Glory need to be further assessed for commercial value before recommending to growers. Table 2.1 summarised the susceptibility of 167 varieties of peaches and nectarines to peach leaf curl disease. Those with low susceptibility chould be incorporated into breeding programmes for stonefruit and assessed in New Zealand conditions.

The seaweed extract, Seagro, did not control peach leaf curl disease on cv. Red Diamond nectarines planted in a randomised complete block design in Palmerston North in 1987. No evidence of fungicidal properties was found in the second year of the study either. However, during this season there were eleven infection periods for leaf curl (figure 2) and both seaweed extract and copper oxychloride treatments were ineffective. In laboratory experiments, the seaweed extracts Response, Ecklonia and

Carpophyllum had no effect on *T. deformans* growth.

A useful, quick laboratory test for comparison of fungal growth on different fungicide/seaweed extract amended PDA plates was the measurement of light absorbance of cell suspensions with a spectrophotometer. A high light absorbance indicated little growth of fungus; and vice versa. This method was quicker than the counting of cells using a haemacytometer.

Growth of the fungus on amended PDA in small jars rather than petri dishes meant that sampling error was reduced and results were more accurate. All cells growing on media in the small jars went into suspension and were measured. On petri plates, counts of cells were made from plug samples. As plug samples were not needed with the small jars an even initial seed layer was not required over a larger surface area.

Potato dextrose agar and 1% peptone were used as the media for the growth of *T. deformans in vitro*. Satisfactory growth was also obtained on whole potato extract but the ease of preparation of the commercial agar mix made it the best option for this study. Other researchers (Cheah and Tate 1986) have also used commercial PDA with similar success for *Taphrina* growth. *T. deformans* grew more slowly on malt agar and peptone and did not grow at all on Czapek media. Acidifying the PDA media to a pH of 3.5 did not significantly affect *T. deformans* growth.

For much of the laboratory work a named isolate of *T. deformans*, from Dr Cheah of the Levin Research Centre, was used. An added complication was the use of a named isolate from the DSIR which later proved to be a yeast. Differences between the LRC and DSIR isolates were found in the petri dish zonal inhibition experiment (3.4.1), comparison of growth on copper-amended PDA (3.4.2) and other pesticide amended PDA (3.5.4) and in the general characteristics of the isolates.

The petri dish zonal inhibition technique was useful for quantifying the effectiveness of some fungicides on *T. deformans*.

Streptomycin, Benlate, and Rovral had little or no effect on the growth of Taphrina deformans in vitro but Saprol inhibited its growth. In field experiments on peach leaf curl where control of the brown rot fungus is required, Saprol is recommended.

Limitations of this study

The isolation of the experimental plot from a whole orchard system meant that attention was focussed on the leaf curl disease and other plant protection or management concerns such as tree vigour, fruit quality, the control of stonefruit blast etc. were not studied. The trees too immature to fruit so the effect of pests and diseases on fruit yield and quality could not be made. Two of the varieties were found to be tolerant to leaf curl but no formal assessment was made of their susceptibility to pests such as aphids, or to other diseases such as powdery mildew, brown rot and bacterial diseases. Problems from such pest organisms were not noticed during the leaf curl assessments.

Trees used in this study were second grade nursery stock. They were poorly shaped (acute crotch angles, no central leaders) and lacked vigour in the first year of the trial. The nectarine variety Red Diamond was also found to be especially prone to bacterial blast. It is not recommended for future research with stonefruit diseases.

Further Research

Further research on both resistant or tolerant peach/nectarine varieties and on the potential use of seaweed extracts is needed. Resistance to leaf curl is only a small part of stonefruit growing. Research must also consider tree vigour, fruit yield and resistance to other pests and diseases.

Growers could improve the effectiveness of their fungicide applications if information on the timing of infection periods for leaf curl was readily available to growers during the season. Research into the most effective method of providing this information for growers is needed.

Red Diamond nectarines are susceptible to bacterial blast and peach leaf curl and are not common in commercial New Zealand orchards. For these reasons they are not recommended for future research on stonefruit diseases.

This study has shown the potential importance of nectarine/peach varieties resistant to diseases such as peach leaf curl to allow development of low pesticide input systems.

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PLATE 2

Red Diamond Nectarines 12 August 1987 (spray application made)

PLATE 3

1988 FIELD TRIAL : CONTROL TREATMENT

September

17 October

PLATE 4

1988 FIELD TRIAL

17 October 1988

Late November 1988

PLATE 5

1988 FIELD TRIAL : RESISTANT VARIETIES

Gordon's Glory Peach mid-September

10 October

Winiata Gold mid-September 1988

PLATE 6

1988 FIELD TRIAL : SEAGRO TREATMENTS

PLATE 7

1988 FIELD TRIAL : COPPER TREATMENT

PLATE 8

1988 FIELD TRIAL : COMPARISON OF COPPER, RESISTANT VARIETIES (GORDON GLORY) AND CONTROL TREATMENTS

APPENDIX

Appendix A

Summary of Varieties Studied for Susceptibility to Leaf Curl

VARIETY	REFERENCE	SUSCEPTIBILITY
Afterglow	2	high
Alexander Early	12	low
Ambergem	2	low
Amsden	3	low
Amsden	8.1,12	moderate
Andross	4	moderate
Autumn Gem	4	high
Babygold#8	4.1	high
Belle of Georgia	2,4	moderate
Best May	2	moderate
Biscoe	4	high
Blake	4	moderate
Burbank Elberta	2	moderate
Camden	4	high
Candor	4	low
Capucci 6	1	low
Cardinal	1	low
Carson	4.1	moderate
Cary Mac	4	high
Cherryred	2	low
Clayton	4	low
Colomba-Rubiette	11	high
Colombia (nectarine)	4	moderate
Com-pact Redhaven	4	low
Correll	4	low
Cresthaven	9	moderate
Davidson Red leaf	2	low
Derby	4	moderate
Desirable	2	low
Dixiland	4	low
Dixired	2,4.1	moderate
Dixon	4	moderate
Earliblaze (nectarine)	4.1	high
Early Elberta	2,(12)	low (high)
Early Fair Beauty	2	moderate
Early Halehaven	2	moderate
Early Jublilee	2	low
Early Red	5	low
Early Red Fre	2	moderate susc. but 2-3 yr old trees had low susc.
Early Redhaven	4	low
Early Sungrand	4.1	high
Early Triogem	2	moderate
Early Vedette	2	low
Elberta	1	low
	2,12	high
	4	moderate
Elberta Late	12	high
Ellerbe	4	low

Elma	3	low
Emery	4	moderate
Eriogen	1	low
Everts	4	moderate
Fairhaven	2	low
Fairtime	4	moderate
False Sunhigh	2	moderate
Fantasia nectarine)	4.1	high
Fay Elberta	2	low
Fayette	9	moderate
Fertile Hale	2	high
Firebrite	4	high
Fireglow	2	moderate
Fisher	2	moderate
Flamecrest	4.1	high
Flamekist nectarine	4.1	high
Flavorcrest	4	high
Flavortop nectarine)	4.1	high
Fort Valley 20	2	low
Fort Valley 110	2	moderate
Fort Valley 993	2	moderate
Frant	8	moderate
Garden State nectarine	2	high
Gem	4	moderate
Gemmer's Late Elberta	2	high
Glohaven	9	moderate
Gold Mine	1	low
Golden Beauty	2	low
Golden Globe	2	moderate
Goldeneast	2	high
Halberta	2	low
Halegold	2	moderate
Halehaven	2	low
Hamlet	4	moderate
Harko nectarine	4.1	moderate
Harvester	4	moderate
Imperio	1	low
Indian Blood	2	low
J.H. Hale	2	low
Jefferson	4.1	moderate
July Elberta	10,2	moderate
Junegold	4	high
Kirkman Gem	4	low
Konservyni Ranni	6	low
Kudesnik	8	moderate
Late Legrand	4.1	high
Lebedev	8	moderate
Lizzie	2	high
Loring	4	high
	2	tolerant or moderate.
Lovell	4	moderate
Madeleine Pouyet	10.1	moderate
Magoscorlup	1	low
Marsun	4.1	moderate
Maygold	10	moderate

McNeely	4	moderate
Mericrest	13	low
Merrill June	2	low
Missouri	2	low
Monroe	4	moderate
Nectar	2	low
Nectared 4 nectarine	4	moderate
	5	low
Nectared 7 nectarine	4.1	high
Nectared-2	5	low
Newday	2	low
Norman	4	high
Novelred	4.1	moderate
Othelle	10	moderate
Ozark	2	low
Pekin	4	low
Pocahontas	10.1	moderate
Podarok Kryma	8	moderate
Prairie Dawn	2	low
Proskauer	3	low
Ranger	4	moderate
Ranii Kubani	7	low
Raritan Rose	2	low
Red Chief (nectarine)	10	moderate
Redcrest	2	low
Redglobe	4	high
Redhaven	4,2	low
	3	moderate
Redskin	4	high
Redwin	10.2	moderate
Regina	4.1	high
Reliance	4.1	moderate
Rio Oso Gem	2	high
	4	moderate
Rose (nectarine)	4.1	high
Rubired	4	low
Sentinel	9	moderate
Sieger	3	moderate
South Haven 20	2	low
Southern Glow	2	low
Southland	4.1	moderate
Springbrite	4	moderate
Springcrest	4.1	high
Springtime	5	low
Stark	12	high
Stark Redgold	5	low
Starking Delicious	2	moderate
Starlite	4.1	high
Sullivan Early Elb.	2	moderate-high
Summer Crest	2	low
Summerset	4.1	high
Sunbrite	4	high
Suncrest	4.1	high
Sunday-Elberta	2	moderate
Sunhaven	3, 5	moderate

Sunhigh	4	high
	2	moderate
Sunqueen	4	moderate
Sunshine	10.2	moderate
Surecrop	4	moderate
Toss Kino	1	low
Triogem	2	low susc. but very susc. in 5 -6 yr old trees).
Troy	4	high
Velvet	4	moderate
Vivid	4	low
Vorgebirgsfirsich	3	low
Whynot	4	moderate
Winblo	4	moderate
Windsor	4	high

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1. Alexandri and Filip (1977)
2. Foster and Petersen (1952) used a susceptibility score scale of 0-5. These were interpreted as 0-1, low susceptibility; 2-3, moderate and 4-5, high susceptibility.
3. Koch (1984)
4. Ritchie & Werner (1981). Fungicides were used in the previous season. Susceptibility was rated on a 0-9 scale, with 0 being no infection and 9 having the entire leaf thickened and puckered. Scores averaging 0-2 were considered of low susceptibility; 2-5 moderateerate susceptibility and 5-9 high susceptibility.

4.1. Ritchie & Werner (1981). No fungicides were used in the previous season

5. Vol'vach (1986)
6. Zagorodnaya (1985)
7. Simakina (1980)
8. Ryabova (1980)

8.1 Ryabova (1980) - only 10 percent leaf infection.

9. Todorovic & Misic (1982)
10. Nitransky (1974)

10.1 Nitransky (1982)

10.2 Nitransky (1983)

11. France (1984)
12. Nesic & Blagojevic (1971)
13. Drake (1984)

Appendix B

Diary of field trial management and observations 1987-88

DATE	ACTIVITY
1987	
15 July	Trees planted with 200g blood and bone fertiliser/tree.
27 July	Trees pruned, prune cuts painted with pruntect. Bacterial infections pruned out. Buds starting to swell.
4 August	Red Diamond nectarine pink/very early bloom
12 August	Sprays applied. Sawdust mulch, 3cm deep, placed at base of all trees. Resistant varieties pink (very early bloom).
25 September	General observations of disease incidence taken. Leaves about 5 cm.
13 October	All trees sprayed with Streptomycin (agrimycin) 0.6 g/l and a wetting agent. Used 30 l for all plots.
18 October	Applied herbicide under trees in the rows. Combination of Buster (10 ml/l), Asulox and a spreader. Weather fine and sunny.
1988	
22 January	Applied Seagro to rep 2 only. 10 ml/l. Calm and fine.
10 February	Applied Seagro to rep 2 only. 10 ml/l. Calm and fine.
1 March	Applied Seagro to rep 2 only. 10 ml/l. Slight wind. Pruned all trees.
10 March	Gordon's Glory fruit ripe.
18 March	Weed control: cleared weeds from tree base with push hoe
23 March	Applied Seagro to rep 2 only. 10 ml/l.
7 April	Applied Seagro to rep 2 only. 10 ml/l. Weed control: cleared weeds from tree base with push hoe
4 July	Copper oxychloride - first spray Start of bud swell, 13°C.
31 July	All Seagro and copper sprays applied to treatments 2-5. Fine, no wind. Used 2.5 l in total.

Copper sprays at Himatangi 1988 (30 km SE of Palmerston North)

14 April	copper oxychloride
3 May	copper oxychloride
25 May	Kocide
26 June	Kocide
29 July	Cupravit
17 October	Bravo^{R}
2 November	Bravo^{R}

Appendix C

MEDIA RECIPES FOR *TAPHRINA DEFORMANS* GROWTH IN VITRO

Acidified PDA (APDA)

2 mls of 25% Lactic acid were added to 800 mls of PDA to obtain a pH of 4. (ie. 0.625ml Lactic Acid per litre).

Cheah

Cheah (1988 pers comm.) used the following media for *Taphrina deformans* ascospore production:

20g Malt powder
2g Z-asparagine
0.6g diammonium hydrogen phosphate
0.3g disodium hydrogen phosphate
0.2g calcium carbonate
10g peptone
1g potassium nitrate
10 mg ferric chloride crystals
5 µg copper sulphate
0.5g potassium dihydrogen orthophosphate
1 litre distilled water

8g sucrose
1g potassium phosphate
0.25g ammonium sulphate
8g D-glucose
1 mg zinc sulphate
1g ammonium nitrate
0.3g magnesium carbonate
1 mg manganese sulphate
10g agar

Dissolve sugars, salts and Z-asparagine in 500 mls of water. Dissolve peptone and malt in 500 mls water, add agar and melt. Mix flasks and cool slightly. Adjust pH to 5.5-6.5 with citric acid. Put into flasks and autoclave for 15 minutes.
Inoculate and place at 21°C for 6 months for ascospore production.

Czapek

Bacto Czapek Dox broth is a liquid medium, nearly neutral in reaction that was designed for the cultivation of fungi and bacteria capable of utilising inorganic nitrogen. Sodium nitrate is the sole source of nitrogen. After autoclaving the final pH of the medium is 7.3. This medium is generally used for the culture of *Pythium* and *Phytophthora* species.

	g/l
agar	15
NaNO3	3
K2HPO4	1
MgSO4.7H2O	0.5
KCl	0.5
FeSO4.7H2O	0.01
Sucrose	30

(Ref. Goodman *et al.* 1967)

Malt agar and peptone

agar	15g/l
malt	30g/l
peptone	5g/l

The malt and peptone were placed in a 2 litre flask and heated in water until they were evenly dispersed. Agar was added and the flasks autoclaved at 15 psi for 15 minutes. These were cooled to about 50°C and poured into petri plates in a Laminar Flow Cabinet.

Whole potato extract media

Base media:
200g potatoes, boiled until soft.
1 litre water

Boiled potatoes were filtered through 2-3 layers of muslin in a funnel and the resulting liquid made up to one litre with distilled water. To remove the solids and provide a clear sample, one half of each potato based mixture was centrifuged at 10 000 rpm for ten minutes.

Supplements to the base media:

Dextrose	12g/l
Lactic acid	1 ml/l

PDMA

potato	200 g
dextrose	20 g
agar	20 g
Marmite	1 g
water	1 litre

PDA

potato	200 g
dextrose	20 g
agar	20 g
water	1 litre

Gibco PDA

Gibco PDA	39 g
water	1 litre

Nutrient media

KH_2PO_4	2.7g
$MgSO_4$	1.2g

dextrose 10 g
KNO_3 2g
Asparagine 2g
agar 20g
water 1 litre.

Appendix D

NOTES ON COMMERCIAL SEAWEED EXTRACTS IN NEW ZEALAND

1. Seagro

Seagro is produced in Hastings, New Zealand and is a registered product of Mineral Additives Ltd. in NZ and Australia. It is made from algit concentrate, a Norwegian seaweed which contains about 128-134 salts and minerals. The kelp is dried in wind tunnels and converted to liquid using a cold process.

Recommended rates for orchards are 9l/3300l water for 1 ha. Foliar spray rates are 1:100 for low volume and 1:400 for high volume applications.

2. Response

Response fertilisers are made by the New Zealand company, Growth Marketing Ltd. The base liquid for response fertilisers is a combination of seaweed extracts of Pterocladia and Ecklonia. Chelates and the applicable NPK for each product are added.

Response seaweed extracts are recommended for use twice before flowering, not closer than fortnightly, and whenever plants are stressed (eg bud formation and fruit set). The dilution rate is 1:300. The manufacturers recommend they are not mixed with metallic sulphates such as magnesium sulphate or complexes of copper sulphate.

3. SM3

SM3 is no longer sold in New Zealand but is still manufactured in the UK. Application rates are 2.75 l/ha to topfruit applied four times from bud burst up to early fruit set. Immediately after harvest 5.5 l/ha is recommended.

Appendix E

Climate data July-September 1987-1988 Palmerston North
(Source: Grasslands DSIR Palmerston North)

	1987			1988		
Date	Rainfall (mm)	Temperature minimum (°C)	maximum (°C)	Rainfall (mm)	Temperature minimum (°C)	maximum (°C)
JULY						
1	1.0	9.2	1.9	0	9.7	0.7
2	0	10.7	.6	2.2	12.5	1.0
3	1.3	12.5	1.3	0	12.9	8.3
4	1.0	12.5	1.4	0	14.4	3.9
5	0	10.6	-0.6	.5	13.4	6.0
6	0	12.3	1.5	1.1	11.6	1.7
7	0	11.9	.5	0	8.3	0.3
8	0	15.1	4.2	0	11.4	.5
9	0	13.6	8.9	0	12.8	5.1
10	0	11.5	0.3	7.2	14.7	2.9
11	6.3	14.0	3.3	9.3	14.6	6.2
12	2.3	12.9	8.7	0	14.3	9.8
13	0.1	13.2	8.8	0	12.4	3.6
14	3.1	13.0	9.1	0	13.5	4.2
15	0.2	12.7	10.2	0	13.0	7.5
16	0.1	11.8	8.8	6.8	12.7	8.2
17	0	14.0	3.7	2.6	12.0	9.3
18	.9	12.5	3.9	5.5	12.0	10.1
19	0	13.4	6.8	0.2	13.0	9.5
20	.2	13.7	2.5	0.5	12.0	4.7
21	0	13.0	5.9	0	13.7	5.6
22	11.9	12.9	4.8	0	14.0	3.7
23	0.2	12.4	3.4	15.6	17.5	5.3
24	0	11.2	4.5	82.1	13.6	9.3
25	2.5	9.8	6.4	2.8	14.1	8.6
26	0	11.9	7.8	5.3	13.7	9.1
27	0	11.3	3.5	17.8	15.1	9.4
28	0	13.6	4.3	0	12.6	7.3
29	0	13.9	3.2	0.8	14.2	2.3
30	0	12.8	-0.7	2.4	14.2	5.5
31	1.7	13.6	0.8	0.3	14.2	9.6

Climate data July-September 1987-1988 Palmerston North
(Source: Grasslands DSIR Palmerston North)

Date	1987 Rainfall (mm)	1987 Temperature minimum (°C)	1987 Temperature maximum (°C)	1988 Rainfall (mm)	1988 Temperature minimum (°C)	1988 Temperature maximum (°C)
AUGUST						
1	0	16.8	4.0	2.8	13.6	7.4
2	0	14.5	9.1	0	13.0	7.1
3	0.6	14.1	7.1	0	14.1	2.7
4	9.4	15.4	5.7	0	14.3	4.1
5	2.7	19.3	10.0	0	14.1	3.8
6	0.4	13.6	9.5	0	14.3	7.6
7	0	15.2	7.1	0	18.4	5.0
8	0	14.9	3.5	17.0	14.9	6.7
9	0	13.8	5.3	4.0	16.9	8.6
10	1.4	12.3	8.8	14.4	14.2	8.6
11	0.5	13.4	8.6	2.8	10.3	8.2
12	0	12.9	3.4	0	10.6	6.1
13	0	14.0	-0.5	0	10.0	0.7
14	0	14.8	1.4	0	15.0	2.2
15	0	14.2	1.6	0	14.3	4.1
16	0.2	13.1	5.1	1.4	13.6	6.8
17	1.1	14.3	7.5	1.6	14.1	8.7
18	0.5	16.3	9.5	1.4	16.5	9.8
19	0	16.0	4.3	0	12.4	4.8
20	0	14.5	1.7	20.1	12.4	5.5
21	0	16.0	7.0	3.1	13.6	8.8
22	8.4	16.3	10.0	0.6	13.3	1.9
23	0.1	15.7	11.2	17.7	14.4	6.6
24	0	16.0	10.8	0	14.5	10.3
25	0.6	18.3	9.8	0.9	9.7	7.4
26	2.3	16.2	10.7	0	11.2	-1.0
27	3.8	14.6	11.4	0	14.4	2.0
28	0	14.7	9.5	0	14.3	0.8
29	0	14.5	6.9	3.0	16.2	4.0
30	0	14.9	2.9	2.7	15.0	9.6
31	0	16.9	4.5	0.3	15.0	7.8

Climate data July-September 1987-1988 Palmerston North
(Source: Grasslands DSIR Palmerston North)

Date	1987 Rainfall (mm)	1987 Temperature minimum (°C)	1987 Temperature maximum (°C)	1988 Rainfall (mm)	1988 Temperature minimum (°C)	1988 Temperature maximum (°C)
SEPTEMBER						
1	0	17.7	3.0	5.5	9.9	7.4
2	5.1	16.4	4.8	24.8	13.0	6.5
3	7.9	14.1	9.9	0	15.9	7.0
4	0.3	15.7	8.7	0.6	12.8	9.3
5	1.0	14.7	8.2	6.7	13.3	9.6
6	0.1	13.0	2.0	0.3	13.2	3.2
7	0.3	12.4	7.3	21.2	15.3	8.4
8	3.0	15.4	6.5	3.4	13.0	8.0
9	1.5	13.9	8.6	0	12.6	-0.5
10	0	15.4	3.3	1.7	13.4	3.9
11	8.3	18.0	6.9	1.3	13.9	9.5
12	9.4	14.6	7.0	0	15.8	10.8
13	5.5	13.4	9.4	20.3	18.0	12.6
14	0.2	13.9	8.6	15.5	14.4	12.8
15	3.0	13.0	3.6	0	14.3	9.3
16	0	13.6	4.3	0	16.3	10.4
17	0.2	15.4	9.6	0	18.6	8.9
18	1.1	14.5	7.9	7.2	19.6	8.9
19	1.1	14.2	2.4	5.5	20.9	12.0
20	0	15.5	5.9	0.1	17.6	12.4
21	0	15.9	5.4	15.0	18.2	11.3
22	5.0	16.8	10.7	0.4	14.5	11.1
23	0.3	15.7	9.0	1.1	14.6	10.1
24	0.3	15.2	8.4	0	17.0	10.8
25	0	15.3	4.8	0	16.3	8.6
26	0	15.6	7.5	1.1	15.0	11.3
27	0.2	20.5	8.1	0.7	16.9	12.3
28	1.8	14.1	10.4	0	18.3	13.4
29	0	16.1	10.2	1.1	18.9	10.5
30	1.5	17.4	11.3	10.5	17.2	12.2