Scoping study on the management of varnish spot in field and hydroponic lettuce

Andrew Watson
NSW Department of Primary Industries

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Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

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VG 03003

Andrew Watson
NSW Department of Primary Industries
National Vegetable Industry Centre
Yanco,
2703.
Phone 02 69 512611
Fax 02 69 512719
Email andrew.watson@agric.nsw.gov.au

Other members of the project team include;
Tony Napier
NSW Department of Primary Industries
National Vegetable Industry Centre
Yanco,
2703.

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MEDIA SUMMARY

There are a number of diseases that affect lettuce. *Sclerotinia*, downy mildew, Anthracnose and *Septoria* spot are examples of fungal diseases. Virus diseases such as big vein, necrotic yellows, tomato spotted wilt virus and lettuce mosaic virus also affect lettuce. Bacterial diseases include bacterial leaf spot and varnish spot. Varnish spot causes brown lesions around the midrib on leaves quite commonly under outside leaves which show no symptoms. This project looked at the awareness of this disease across the lettuce industry in Australia through a survey and industry contact. The main key points of the project were;

- The disease was reported in all states of Australia.
- Growers surveyed were able to recognise the disease and a large proportion had the disease present on there properties most only occasionally.
- Some growers reported that they only had minor losses due to this disease; others had whole blocks of lettuce rendered unmarketable.
- Losses ranged from 0-20% in crop figures or up to $200,000 in monetary figures.
- Growers generally thought that this disease was mainly seen in spring.
- Most of the respondents considered that further research should be carried out on this disease.

Varnish spot may be more noticeable on lettuces harvested for processing. This is because the outside leaves which may be symptomless are removed and the leaves below may show the typical varnish spot lesion.

The bacterium that causes varnish spot belongs to the group of bacteria that are associated with browning of cut lettuce. They are soil borne and may infect lettuce through water splash from soils that contain the bacterium. Other possible methods of infection could be seeds, transplants and insects, but these need further investigation. Varieties may be variable in their susceptibility to varnish spot.

There are no control methods for this disease. Therefore it is recommended that detection methods for the bacterium and varietal resistance should be investigated. The level of the bacterium in soil and water should be further investigated and the effectiveness of various application times and types of copper sprays should also be studied.

TECHNICAL SUMMARY

Varnish spot is a bacterial disease that causes brown lesions around the midrib on lettuce (*Lactuca sativa* L.) leaves quite commonly under outside leaves which show no symptoms. Varnish spot of lettuce is caused by *Pseudomonas cichorii*. The disease has been found in other countries including Italy, Turkey, Brazil, North America and is common in Australia. This project was established to find the awareness and seriousness of this disease across the lettuce growing regions of Australia.

Growers and others in the industry were visited, met with at industry information nights and meetings or called by telephone to obtain information on varnish spot. In response it was found that the majority of growers had seen the disease and were able to distinguish it from other bacterial rots such as those caused by *Erwinia*. All states had growers that were affected by varnish spot. It was previously thought that only some states had this disease. Most growers surveyed had minor losses from varnish spot but some growers recorded large losses such as $200,000, $145,000 and $40,000. These growers were in three different states. It appears that varnish spot can affect a lettuce sporadically or totally wipe out a planting. One hydroponic grower had also recorded heavy losses as a result of infection by *Pseudomonas cichorii.*

Processors reported that it was an issue for lettuce processed for hearts, with one processor recording a 3% loss from this disease. Seed companies agreed that most states had the disease with one company having problems with varnish spot on some varieties in 2005.

Previous research on this disease shows that it can infect through stomata and epidermal hairs. The bacteria can survive in lettuce residue so crop rotation has been suggested as a control option. But reports through this project have indicated that varnish spot has occurred on blocks that have not had lettuce for up to three years. Other sources of inoculum include seeds, transplant and insects. Some growers in the survey considered water sources as a possible source of bacteria; this has been supported with overseas information. Other hosts of *P. cichorii* include chicory cabbage, cauliflower, celery tobacco and endive. Numerous weed hosts could also exist.

A possible management plan for varnish spot would include the following;

- Water used for seedlings should be tested for varnish spot bacteria. Transplants should be inspected for signs of disease and infected transplants destroyed.
- Irrigation should be carried out to minimize leaf wetness duration and especially reduced to a minimum within three weeks of harvesting.
- Fields are rotated for 4-5 years between lettuce crops or other hosts of the pathogen causing varnish spot.
- Hosts should not be planted in adjacent fields.
- Plant less susceptible lettuce varieties.
- Lettuce should be planted in fields with well-drained soil and good air movement to promote rapid drying.

Future research on this disease would need to;

- Investigate and develop inoculation methods to reproduce varnish spot artificially for use in field and laboratory trials.
- Developing a quick diagnostic test for *P. cichorii* and other fluorescent pseudomonads that contribute to lettuce browning.
- Investigate varietal differences to varnish spot.
- Investigate through trials the efficacy of copper treatments with the aim of controlling varnish spot but also to reduce any sensitive reactions to the copper compounds.
- Examine weeds for their potential as a source of *P. cichorii* inoculum.
- Examine seeds, transplants, alternate hosts and insects as possible sources of inoculum.
- Investigating and develop water testing methods for *P. cichorii.*

INTRODUCTION

Lettuce production is worth A$122million (2001)(ABS Agstats). There are a number of diseases that affect lettuce. *Sclerotinia*, downy mildew, Anthracnose and *Septoria* spot are examples of fungal diseases. Virus diseases such as big vein, necrotic yellows, tomato spotted wilt virus and lettuce mosaic virus also affect lettuce. Bacterial diseases include bacterial leaf spot and varnish spot. The main symptom of varnish spot is brown discoloration of the midrib or around the midrib.

Varnish spot can be found all year but seems to be most serious in the late winter and spring months. Lettuce with varnish spot may be rejected at harvest or harvested then transported to markets without any knowledge of the disease, only to be rejected on delivery.

Typical symptoms are shown in the pictures below.

Figure 1 **Figure 2**

Lettuce from Hay in 1999, associated with a severe outbreak of varnish spot.

Figure 3 **Figure 4**

Figures 3 and 4 are of a lettuce found in Cowra in 2004 where *Pseudomonas* bacteria were isolated. Note that the symptoms were slightly different symptoms of those in Figures 1 and 2.

Varnish spot is caused by bacteria belonging to the genus *Pseudomonas*. The disease appears close to harvest where the crop may look healthy until the lettuces are harvested. The spots may not be visible from the outside of the lettuce but when the

outer leaves are removed the brown spots are found, often along the midrib of inner leaves. These small lesions can expand rapidly from discrete spots to rotting. Other bacteria may infect through the damaged tissue and cause rotting of the whole heart. Unfortunately whole blocks of lettuce can be affected. Symptomless, but infected heads in combination with secondary rotting bacteria can develop rapidly in storage.

Varnish spot should not be confused with soft rot caused by another bacterium called *Erwinia sp* which may also occur on lettuce, usually every year, especially as summer temperatures approach. *Erwinia* enters the plant through injuries eg frost or spray damage causing heads to go slimy and unmarketable. Varnish spot can infect lettuce without these injuries.

How does varnish spot infect and cause disease?

A disease called varnish spot was found in California in 1977 (Grogan 1977). The disease was found to be caused by *P. cichorii.* The pathogen was isolated from soil and root samples from the field. The study also found that this disease was found only on sprinkler irrigated fields.

The Compendium of Lettuce Diseases (Davis *et al.* 1997) suggests that the disease can be soil borne and often associated with lettuce debris, but also suggests that seeds, transplants, alternate hosts and insects may also be sources of inoculum. Seeds and insects may be an important source of the bacteria in Australia. Seed health is an important issue for growers. Insects such as aphids and thrips, which are common inhabitants of lettuce hearts, could be sources of the bacteria.

The infection process is not that clear. Insects may cause injuries through which the bacteria may enter the plant. It is most likely that rain or overhead irrigation contributes to the disease. Water reservoirs are a possible source of bacteria. The bacteria may be introduced through water used for irrigation, regular sprays or by soil splashed by rain. The bacteria are spread by rain and wind.

Research has shown that *P. cichorii* was observed in stomata of leaves of the middle part of lettuce. It was also observed that there was no browning associated with the presence of the bacteria. It was also suggested that *P. cichorii* first invades the lettuce through stomata and later multiplies in the intercellular spaces of the epidermis and then progressed to colonise the intercellular spaces of the mesophyll (Hikichi *et al.* 1996).

Other work has shown that *P. cichorii* was present in seed (Ohata *et al.* 1982) and was able to be removed from seed by heat treating seed (Ohata *et al.* 1982). Unwounded lettuce tissues were readily infected through the stomata and epidermal hairs (Shirata *et al.* 1982). *P. cichorii* can be spread by aerosols when water is splashed onto soils and plants (Hikichi *et al*. 1996).

Cold storage implications for varnish spot.

As mentioned previously bacteria of the *Erwinia* species cause soft rot of lettuce. One important difference between *Pseudomonas* and *Erwinia* is that the latter only grows slightly at 5^0C whereas *Pseudomonas* species have the ability to rot lettuce at temperatures close to 0^0C (Nguyen-The and Prunier 1989).

Control recommendations.

Potassium sulphate applied as a fertiliser, has been reported to increase disease (Bleyaert *et al.* 1999), and treatments with copper products applied every 10-15 days after swelling of the heart was found to improve disease control (D'Ascenzo *et al.* 1997). Bordeaux mixture and copper oxychloride were found to be more effective than copper hydroxide in reducing varnish spot in field trials in Italy (Bazzi and Cazolari 1984).

Recent report of varnish spot.

A new disease was reported on lettuce in Turkey in 2002, the disease was caused by *P. cichorii*. There was no proof but it was speculated that contaminated seeds and/or transplants were the source of the introduction of the pathogen to the region (Aysan *et al*. 2003).

The bacterium also causes leaf spots in ornamental plants such as Geraniums and Chrysanthemum.

Project VG 98083 "A study of post harvest bacterial rots and browning in lettuce and the development of control methods"

A previous project VG 98083 looked at the effects of the *Pseudomonas* group on browning in cut lettuce. However, some of the information was relevant to varnish spot. Two of the aims of the project were to monitor the levels of fluorescent pseudomonads (the group that *P. cichorii* belongs to) in the soil and to measure their seasonal abundance. In the Werribee region, fluorescent pseudomonads were found higher in winter and the lowest levels in summer. In the Gippsland region levels of fluorescent pseudomonads were found to be significantly higher in spring 2001 than summer 2001. Their investigations found that fluorescent pseudomonads in soils of lettuce growing regions varied over the seasons and varied between crop types. They also found that lettuce waste left after harvest contained high percentages of *Pseudomonas* species of bacteria.

VG 03003 "Scoping study on the management of varnish spot in field and hydroponic lettuce" developed from a research application that was submitted to look more closely at this disease. This project is an extension/ evaluation study to find the awareness and extent of the problem in all lettuce growing areas.

An extension was requested till mid 2005 as 2004 had minimal disease and also to fit in with the third lettuce conference at Werribee.

VARNISH SPOT OUTBREAKS-CASE STUDIES.

CASE STUDY ONE

State-New South Wales
Lettuce production type-Field
Type of lettuce-Iceberg.
Previously grown on the same block- no lettuces for 3 years.
Irrigation type-Furrow.
Market-Fresh market.
***Pseudomonas cichorii* confirmed**-Yes
Loss-$30,000

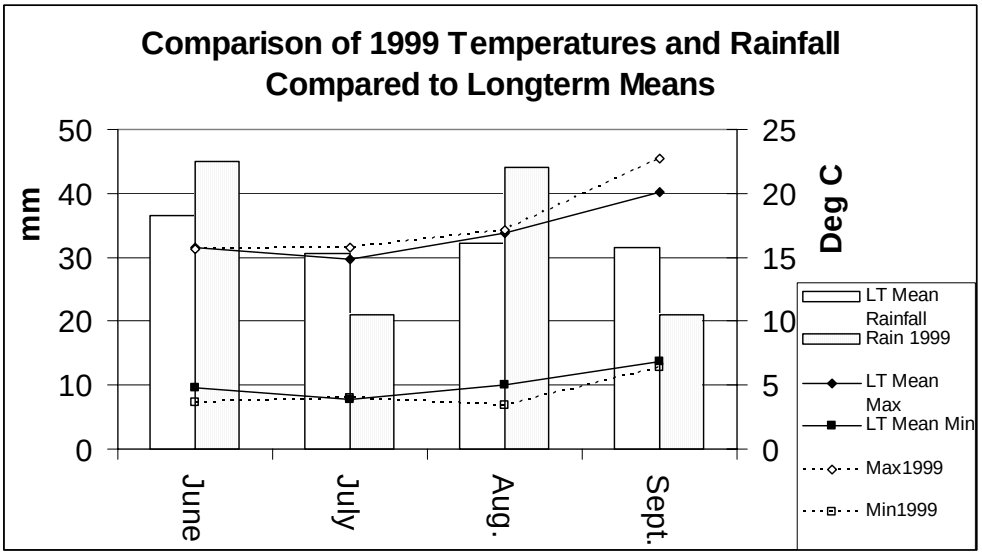
The initial contact by the author with the disease was in Hay in September 1999. A grower had whole plantings affected with other growers having sporadic outbreaks.

Lettuces had become infected in the field and some had gone slimy during transit or in some cases during cold storage. The crop was growing on soil that had not had lettuces grown in it for 3 years previously. More importantly the disease was associated with furrow irrigation whereas it is more generally considered as being more associated with overhead irrigation.

Plant samples were collected and the bacteria *Pseudomonas cichorii* and *Pseudomonas marginalis* were isolated. The identification of *P. cichorii* was able to confirm that the disease was varnish spot. As *P. marginalis* was isolated another leaf spot called marginal leaf spot was also identified as being present. This organism is very widespread and is often associated with post harvest breakdown of vegetables.

Weather conditions were examined for the period to see if it could explain the problem. The average maximum temperature for September was 22.7^{0}C which was higher than the long term average of 20.1^{0}C. This observation matches many of the comments from survey respondents that higher spring temperatures can result in the higher incidence of this disease. This in combination with above average rainfall in September could explain the occurrence.

Another observation on this outbreak was that growers discovered that even when the lettuces were placed in cool storage the rot became worse.



CASE STUDY TWO

State-Queensland
Lettuce production type-Hydroponic
Type of lettuce-Iceberg.
Irrigation type-Hydroponic
Market-Fresh market.
***Pseudomonas cichorii* confirmed**-Yes
Loss- Up to 50% of the crop.

This grower lost 50% of his lettuce crop and had the organism causing the disease identified as *P. cichorii.* His comments were that the problem occurred when there was a lot of moisture in the atmosphere due to dew or rain followed by heat or humidity.

CASE STUDY THREE

State-Victoria
Lettuce production type-Field
Type of lettuce-Iceberg
Previously grown on the same block- no lettuces for 3 years.
Irrigation type-Overhead
Market-Processing.
***Pseudomonas cichorii* confirmed**-No
Loss-$40,000 per year

In one block that had not grown lettuce for 3 years, there was 50% infection. The grower's comments were that it was worse in Greenway, worse around weeds and weedy riser lines. It was also worse as heads were allowed to get heavy and appeared to be related to pinking of stored lettuce.

The main bacterium usually associated with varnish spot was not confirmed at this site, organisms found included *Pseudomonas marginalis* and *Pseudomonas putida*. The normal causal organism *P. cichorii* was not detected. A photograph of an affected lettuce typical of those found at the site is shown below. The brown marking was not as clear on the midrib as the normal varnish spot symptom.

Isolating the correct causal organism can be difficult so the non detection of *P. cichorii* may be considered an anomaly however the symptoms did not appear as severe as varnish spot observed previously. It's quite possible that these organisms could have been contributing to this symptom expression.

Variety trial

A variety trial was conducted on this site by a third party and their response was that of the 15 varieties replicated twice in the July transplanted trial four varieties recorded moderate for varnish spot when assessed. The four varieties were Grenadier, Patagonia, RZ 45-98 and Greenway. All are Salinas or (Salinas x Vanguard) types and none of the straight Vanguard types recorded varnish spot.

SOME SELECTED COMMENTS FROM THE SURVEY.

"We are a reseller of fertilisers, chemicals, seeds and agronomy to growers. (It) affects many of our growers and often wipes out a planting in winter. Lettuce rather sensitive to copper sprays so between a rock and hard place." Reseller- Western Australia.

"Varnish spot is not a big concern in the Lockyer Valley." Reseller- Gatton, Queensland.

"Varnish spot not a major concern in my limited experience" Grower- Bacchus Marsh, Victoria.

"We are a research company developing new varieties. Varnish spot is occasionally damaging and we would like to have a variety sensitivity testing protocol." Seed Company-Victoria.

"I have seen it in all growing regions around Australia." Seed Company Representative, New South Wales.

"When found in the crop it is very devastating on the crop." Agronomist, Murray Bridge (South Australia) after referring to a crop that was lost at a cost of $145,000.

"We do lettuce processing; this is a significant problem for us." Processor reflecting an estimated 3% loss.

"Not such an issue on this farm at this stage-most years see some ……..I always believed it came from infected water in dams." Grower-Queensland.

"I see it mainly in autumn or spring when the temperature is around 19-21 ^{0}C" Grower -Western Australia.

"I use low rates of copper oxychloride as a tank mix with each spray applied; I don't have a problem with varnish spot". Grower-Western Australia.

"I have more of a problem in my lettuces that go for processing compared to those that go for the fresh market" Grower –Victoria.

DISCUSSION

It was encouraging to see that growers were aware of the disease called varnish spot caused by *Pseudomonas cichorii* and were able to distinguish it from the other bacterial rot caused by *Erwinia*. They often reported that they didn't see the disease until they removed a few outside leaves. It was unfortunate to see that some growers received large losses from this disease (one a loss of $200,000 and another at $145,000). The case studies highlighted some interesting anomalies. For example case study one highlighted that the varnish spot occurred on lettuce that received no overhead irrigation but did receive rain around the critical period ie close to harvest. Also the ground had not been used for lettuce for three years previous. Weeds have been identified as carriers of the bacterium in Japan (Ohata *et al.* 1982). The carrying potential of weeds in Australia has not been identified.

The project highlighted that varieties vary in their resistance to the disease with a variety trial at the site of case study three showing variability in disease expression. An independent comment from a seed company representative stated that they would like a sensitivity testing protocol developed for their varieties and the different reactions to varnish spot.

One reply from a processor gave a figure of 3 % losses at the factory due to varnish spot. As the figure is "at the factory" it would be assumed that some lettuces affected would have been culled out by growers so the actual on-farm figure would have been higher. The problem as mentioned before is that varnish spot may not be detectable until after the outside leaves are removed. With normal fresh head lettuce many would go to market with undetected varnish spot. However lettuces that go to processors as hearts have a higher frequency of varnish spot and therefore the highest losses.

There were some regional differences that appeared during the survey. In the Perth Metropolitan area which is north of Perth, varnish spot is not as common as the South of Perth. Two reasons for this could be the sandier soils of the former region and also the common occurrence of applying metham sodium, a soil fumigant.

Australian research on the source of *Pseudomonas cichorii.*

Through work on the bacterium through Project VG 98083 "A study of post harvest bacterial rots and browning in lettuce and the development of control methods", *P. cichorii* was commonly isolated from soil. Previous to this the fluorescent pseudomonads had been found in soil, usually associated with organic matter (Rovira and Sands 1971).

The infection sources of fluorescent pseudomonads have been documented as plant debris, water, seeds and soil. It is unknown if insects may play a role in infection. A number of respondents indicated that they considered water as a source of the bacteria. If this is the case then it may be possible for the disease to develop in the field where overhead irrigation is used or also on seedlings in nurseries that may be irrigated with infected water. As *P. cichorii* is found in soil how its actual mode of transmission/infection is not clear.

Controlling varnish spot with copper.

As mentioned in the introduction copper has been trialled in other countries as a control option. A surveyed grower considered that he did not have the disease because of applications of copper oxychloride. Unfortunately some growers find lettuce sensitive to copper and so therefore application of copper using different types, rates and time of application to reduce damage should be considered as a research priority. There are no curative chemical control methods for these bacteria. Any research on copper would also benefit management of bacterial leaf spot.

Conclusion

This project has highlighted that this disease is found in each state. It can be damaging, often severely. Previous work has shown that the bacterium is found in soil. Infection is possible through stomata. Lettuce produce leaves from the outside towards the inside, that is the inside leaves are the youngest which has implications on not only how the inner lettuce leaves are infected but also the efficacy of any chemical treatment that may be applied to the outside of the heart. It may be carried in water and may be transferred through seedlings. An important task would be to develop a rapid test for this bacterium, examine water supplies and soils used for lettuce production.

Could other bacteria produce similar symptoms such as in case study 3 where *Pseudomonas marginalis* and *Pseudomonas putida* were found in a similar lesion as varnish spot? Other investigations that would benefit the lettuce industry would be to develop infection methods and to study the potential of the copper compounds to control this problem. Also to evaluate varietal differences that exists to varnish spot.

This project has shown the sporadic but sometimes devastating nature of this disease. Australia consists of lettuce growing regions that cover a range of climates. Lettuce are grown in different times of the year, using different varieties and different growing methods, however varnish spot appears to cover all these regions.

A management plan for varnish spot should include the following.

- Water used for seedlings should be tested for varnish spot bacteria. Transplants should be inspected for signs of disease and infected transplants destroyed (Do we have this ability without a quick test? Also could it be seed borne?).

- Irrigation should be carried out to minimize leaf wetness duration and especially reduced to a minimum within three weeks of harvesting (What about case study one with no overhead irrigation?).

- Fields are rotated for 4-5 years between lettuce crops or other hosts of the pathogen causing varnish spot (other hosts include cabbage, cauliflower, celery, chicory, chrysanthemum, endive and tobacco). Weeds also may harbour the bacterium in Australia

- Hosts should not be planted in adjacent fields.

- Plant less susceptible lettuce varieties (do we have this information for Australia?).

- Lettuce should be planted in fields with well-drained soil and good air movement to promote rapid drying (are Australia's planting densities too high?).

TECHNOLOGY TRANSFER

This project was essentially a technology transfer project however other activities included.

- Article in Good Fruit and Vegetables. April 2004, 14: 14
- Article in the National Vegetable Industry Centre's Vegie Bites.
- Poster at the 3rd Lettuce Conference in Werribee, 2005.

RECOMMENDATIONS

Future research on this disease would need to;

- Investigate and develop inoculation methods to reproduce varnish spot artificially for use in field and laboratory trials.
- Developing a quick diagnostic test for *P. cichorii* and other fluorescent pseudomonads that contribute to lettuce browning.
- Investigate varietal differences to varnish spot.
- Investigate through trials the efficacy of copper types (there are a number of different formulations) with the aim of controlling varnish spot but also to reduce any sensitive reactions to the copper compounds.
- Examine weeds for their potential as a source of *P. cichorii* inoculum.
- Examine seeds, transplants, alternate hosts and insects as possible sources of inoculum.
- Investigating and develop water testing methods for *P. cichorii*.

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