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Plant disease record

A stem canker disease of olive (*Olea europaea*) in New Zealand

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Abstract A disease complex, with symptoms that include stem cankers and tip die-back, is reported in New Zealand olives (*Olea europaea* L.). Bacteria from stem cankers of olive were consistently isolated as pale lemon-yellow colonies on King's medium B. On the basis of microbiological, molecular, and pathogenicity tests the bacterium isolated was identified as *Xanthomonas* sp. The bacterial isolates allowed verification of Koch's postulates on young olive trees. As a result of our findings we suggest that the stem canker on olive is caused by *Xanthomonas* sp. In addition a fungus identified as *Fusicoccum luteum* was consistently isolated from stem cankers and tip die-back. In inoculated plants *F. luteum* occasionally formed cankerous symptoms though not as aggressively as *Xanthomonas* sp. At this stage it is not clear what role *F. luteum* has in primary infection.

Keywords olive; stem canker; tip die-back; *Xanthomonas* sp; *Fusicoccum luteum*

INTRODUCTION

Olive (*Olea europaea* L.) is one of New Zealand's emerging crops with olive groves becoming established from Northland to Central Otago. There are currently an estimated half a million trees planted (Stanley pers. comm.), with the most widely planted varieties being 'Manzanillo' and 'Barnea'. In New Zealand the olive crop is grown from grafting material imported from a variety of countries, including Israel and Australia.

In 1996, a cankerous disease of the olive variety 'Barnea' (from Hikurangi, North Auckland) was observed on 4–5-year-old trees. The disease was subsequently detected in Nelson in 1999. Initial disease symptoms on stems were brown necrotic patches with yellow chlorotic borders. As the disease progressed, the necrotic tissue increased in depth, cracks appeared in the stems, and the symptoms progressed into swollen cankers (Fig. 1). Side shoots girdled by necrotic tissue and tip die-back were observed (Fig. 2). The sites of infection on the diseased olives were related to pruning wounds and shoot nodes. This study describes the disease symptoms, isolation, and characterisation of the causal pathogens.

MATERIALS AND METHODS

Bacterial isolations

Small tissue pieces from stem lesion margins and surfaces of cankers were removed aseptically, ground in bacteriological saline (0.85% w/v NaCl), and left at room temperature (c. 20°C) for 10 min. The suspensions were streaked onto King's medium B (KB) (King et al. 1954) and incubated at 26°C. Bacterial colonies growing from the suspensions were re-streaked onto KB to obtain single colonies.

Isolates were routinely grown on KB at 26°C and stored at 4°C for up to 2 weeks. For longer-term storage bacterial strains were stored in freezing medium at –80°C (Birch et al. 1997). All isolates used in this study are listed in Table 1.

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Fig. 1 Five-year-old stem of olive (*Olea europaea* 'Barnea') exhibiting cankerous symptoms.

Fungal isolations

Segments of bark covering the surface of cankers and dead twigs were stripped off using flame-sterilised scalpels. Small pieces of the underlying tissue were excised, plated onto potato dextrose agar containing 50 mg/litre streptomycin sulphate (PDAS) and incubated at room temperature (c. 20°C). Fungal colonies growing from the tissue were plated onto PDA (without streptomycin) and incubated under near-UV light at c. 20°C for 14 days after which the fungi were identified.

Pathogenicity tests

Before inoculation, young olive trees were kept in a saturated environment (c. 100% RH, c. 24°C) controlled by an electronic wetness sensor for 48 h. All fungal and bacterial inoculations were performed on the olive varieties 'Barnea', 'Manzanillo', and 'J5'. The fungal and bacterial test isolates were also co-inoculated into the olive varieties to determine if the disease symptoms could be attributed to a combined effect of the bacteria and fungi isolates.

After inoculation plants were kept in a saturated environment for 48 h (c. 100% RH, c. 24°C) and then grown under normal greenhouse conditions. Disease symptoms at the inoculation sites were assessed weekly. Isolations were made from the inoculated sites after 3 and 6 months.

Bacterial inoculations

Young olive trees were inoculated with the bacterial test isolates by wounding the stems by pricking with 20-gauge sterile needles. A syringe was used to inoculate the wounded sites with a bacterial suspension containing c. 10^7 colony forming units (cfu) per ml, in sterile bacteriological saline. Control sites were inoculated with sterile bacteriological saline.

Fungal inoculations

Fungal cultures were inoculated into young olive trees by making small longitudinal cuts in the stem and branches with a flame-sterilised scalpel and inserting small amounts of mycelium into the wounds. The inoculated sites were covered with tape. No mycelium was inserted into wounded control sites.

Cultural and biochemical tests

Gram tests using the methods of Cerny (1976) and Gregersen (1978) were performed on fresh cultures of the bacterial isolates. The presence of flagella was determined by transmission electron microscopy

Fig. 2 Olive (*Olea europaea*) branch exhibiting symptoms of tip die-back.

(TEM) examination. Observations on colony morphology were made on KB, sucrose nutrient agar (SNA), and glucose yeast extract calcium agar (GYCA) (Dye 1962). Growth was tested on nutrient agar containing 0.1% and 0.02% tri-phenyl tetrazolium chloride (TTC) (Sands 1990). The ability to grow at 30, 33, and 36°C was also investigated using the methods of Dye (1962).

The methods described by Dye (1962) were used to test for milk proteolysis, oxygen requirements, lipolysis of "Tween 80", gelatin hydrolysis, presence of oxidase, utilisation of asparagine, acid production from carbohydrates (arabinose, glucose, mannose, erythritol, and salicin), and NaCl tolerance. These tests have been used to characterise and distinguish the species of the genus *Xanthomonas* (Schaad & Stall 1988).

Molecular characterisations

Repetitive extragenic palindromic-polymerase chain reaction (Rep-PCR) fingerprinting

Rep-PCR fingerprints were amplified from the *Xanthomonas* isolates using BOXA and ERIC primers described by Louws et al. (1994). Genomic DNA was extracted using the method of Rudner et al. (1992). Two PCR amplifications were performed for each bacterial isolate. Amplification products (10 µl) were analysed by electrophoresis in a 1.6% w/v agarose gel and detected by staining with ethidium bromide (Sambrook et al. 1989). Rep-PCR banding patterns were matched by eye, and similarity coefficients for pairwise comparisons between isolates were calculated using the following formula (Nei & Li 1979):

$$F = 2n_{xy}/(n_x + n_y) \times 100$$

where n_x and n_y are the number of bands from populations x and y respectively, and n_{xy} is the number of bands shared by strains x and y.

Analysis and sequencing of 16S rDNA

The 16S rRNA gene of isolates 36A from olive was amplified by PCR using primers 16F27 and 16R1525 described by Hauben et al. (1997). The PCR products were further purified using a Wizard® (Promega) DNA clean up kit according to the manufacturer's protocol. A 16S rDNA sequence was determined by using 16F27, 16R1525, 16F530, and 16R1087 primers (Hauben et al. 1997) on the PCR product. PCR products were sequenced by using a Taq Dye Deoxy terminator cycle kit (Applied Biosystems) and a model ABI377 automatic sequencer. Sequence data were analysed by calculating the distance matrix using the Kimura-2 parameter method. A dendrogram estimating the relationships among strains was calculated with the UPGMA (unweighted pair group method with arithmetic averages) using the Phylip interface available on the ribosomal database project (http://rdp.cme.msu.edu/html/analyses.html).

Table 1 Bacterial strains. (ICMP = International Collection of Micro-organisms from Plants, Manaaki Whenua, Landcare Research New Zealand Ltd.)

Isolates		Host	Origin
36A	From stem canker	*Olea europaea* 'Barnea'	Northland
36B	From stem canker	*Olea europaea* 'Barnea'	Northland
36C	From stem canker	*Olea europaea* 'Barnea'	Northland
99XA	From stem canker	*Olea europaea* 'Barnea'	Nelson
99XB	From stem canker	*Olea europaea* 'Barnea'	Nelson
99XC	From stem canker	*Olea europaea* 'Manzanillo'	Nelson
99XD	From stem canker	*Olea europaea* 'Manzanillo'	Nelson
96.04	*Xanthomonas campestris* pv. *pruni*	*Prunus persica*	Otago
95.109	*X. campestris* pv. *pruni*	*Prunus armeniaca*	Otago
9369	*X. c* pv. *populi*	*Populus* × *generosa*	ICMP
25*	*X. c* pv. *citri*	*Citrus sinensis*	ICMP
Xcj-B	*X. c* pv. *juglandis*	*Juglans regia*	Otago
9367	*X. c* pv. *populi*	*Populus* × *generosa*	ICMP
7383	*X. c* pv. *vesicatoria*	*Lycopersicon esculentum*	ICMP

*Because of quarantine restrictions DNA was isolated under containment and used for Rep-PCR fingerprinting only.

RESULTS

Isolation and biochemical tests

The identity of the bacterial isolates was confirmed as *Xanthomonas* sp. on the basis of cultural, biochemical, and molecular characteristics. Small, pale, lemon-yellow colonies were consistently isolated from the stem cankers following 48 h incubation at 26°C on KB. All isolates consisted of motile Gram-negative rods each with a single polar flagellum, and were strict aerobes. Isolates did not grow in a medium containing asparagine as the sole nitrogen and carbon source and growth was inhibited by 0.1% TTC. Mucoid growth was observed on GYCA and 5% SNA. All isolates were oxidase negative. Positive reactions were observed for hydrolysis of gelatin, lipolysis of Tween 80, proteolysis of milk, and presence of catalase. All isolates produced acid from arabinose, glucose, mannose, and erythritol. Except for strains 9369 and 9367, acid was not produced from salicin.

The identity of the fungal isolates was confirmed as *F. luteum* on the basis of cultural characteristics including a typical yellow staining of the PDA medium, which turned a dirty purple with age, and on conidial form and dimensions (Pennycook & Samuels 1985). *F. luteum* was consistently isolated from both the stem cankers and the tip die-back.

Table 2 Pathogencity of test isolates. (+++ = severe necrosis; ++ = moderate necrosis; + = slight necrosis; and – = no reaction.)

	Olive varieties		
Isolate	Barnea	Manzanillo	J5
36A	+++	++	+
36B	+++	++	+
36C	+++	++	+
96.04	–	–	–
9369	–	–	–
9367	–	–	–
Fusicoccum luteum	+	++	+
Saline	–	–	–
Co-inoculation with 36A and *F. luteum*	++	++	+

Pathogenicity tests

The isolates of *Xanthomonas* sp. from the diseased olive trees caused brown necrotic patches with yellow chlorotic borders and eventually swollen cankers in stems of all the inoculated olive varieties. Symptoms were first apparent 6 weeks after inoculation. *Xanthomonas* sp. was re-isolated from the inoculated trees and was found to be identical to the isolates from the original diseased olive trees in all characteristics. *Xanthomonas* sp. was consistently re-isolated at 3 and 6 months after inoculation and caused varying levels of necrosis depending on the olive variety (Table 2). The most extensive necrosis was observed on 'Barnea', whereas on 'Manzanillo' and 'J5' the symptoms ranged from moderate to slight necrosis. Strains of *X. campestris* pv. *pruni* and *X. campestris* pv. *populi* did not provoke disease symptoms in any of the olive varieties tested. No disease symptoms developed in any of the control plants inoculated with bacteriological saline.

Table 3 Similarity coefficients between isolates of *Xanthomonas* sp. calculated with BOXA and ERIC fingerprint data. (Values on the upper right are the similarity coefficients obtained from the BOXA fingerprint data and the values on the lower left are the similarity coefficients obtained from the ERIC fingerprint data.)

	Olive strains						Control xanthomonads				
Isolate	36A	36B	36C	99XA	99XC	99XD	96.04	95.109	25	9369	Xcj-B
36A		100	100	97	83	83	32	32	19	30	44
36B	100		100	97	83	83	32	32	19	30	44
36C	100	100		97	83	83	32	32	19	30	44
99XA	91	91	91		86	86	13	13	19	8	16
99XC	85	85	85	81		100	26	26	19	28	15
99XD	85	85	85	81	100		26	26	19	28	15
96.04	24	24	24	17	20	20		100	52	55	27
95.109	24	24	24	17	20	20	100		52	55	27
25	19	19	19	19	26	26	52	52		55	33
9369	23	23	23	16	20	20	82	82	52		27
Xcj-B	14	14	14	21	14	30	40	40	15	32	

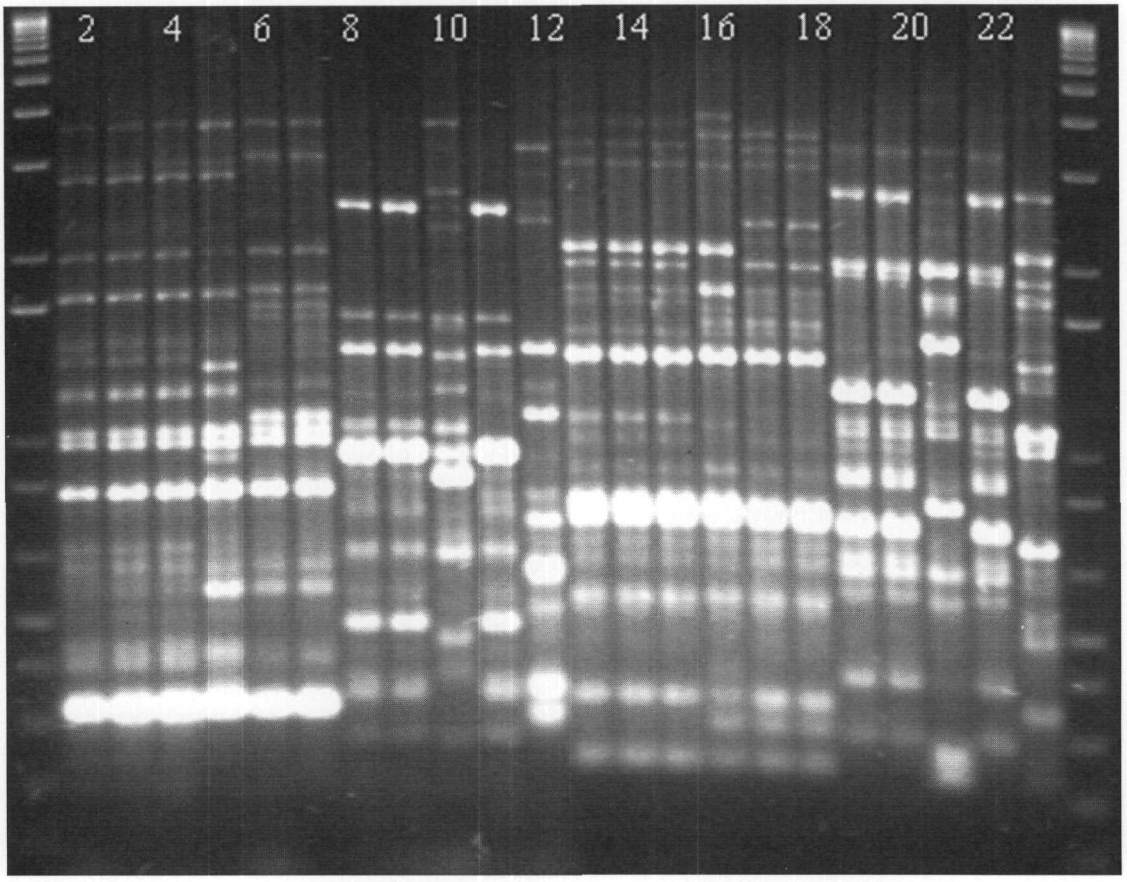

Fig. 3 Rep-PCR fingerprinting patterns from genomic DNA of *Xanthomonas* isolates. Lanes 2–12, ERIC-PCR fingerprints: 1, 1 kb ladder plus (Life Technologies Inc.); 2, 36A (olive); 3, 36B (olive); 4, 36C (olive); 5, 99XA (olive); 6, 99XC (olive); 7, 99XD (olive); 8, 96.04 (peach); 9, 95.109 (apricot); 10, 25 (orange); 11, 96.04 (peach); 12, Xcj-B (walnut). Lanes 13–23, BOXA-PCR fingerprints: 13, 36A (olive); 14, 36B (olive); 15, 36C (olive); 16, 99XA (olive); 17, 99XC (olive); 18, 99XD (olive); 19, 96.04 (peach); 20, 95.109 (apricot); 21, 25; 22, 96.04 (orange); 23, Xcj-B (walnut); 24, 1 kb ladder plus (Life Technologies Inc.).

The fungal cultures isolated from the inoculated olive trees were identical to those cultures isolated from the original diseased olive trees. At most of the sites inoculated with *F. luteum* there was only a weak pathogenic reaction, but small, raised lesions developed on two inoculated sites on 'Manzanillo' and one on 'J5'. Cultures of *F. luteum* were obtained from the majority of the inoculated sites, including some with little or no visible pathogenic reaction. At some sites the fungus penetrated beyond the immediate site of inoculation, but die-back symptoms were not observed in the inoculated material. Co-inoculation of *Xanthomonas* 36A and *F. luteum* did not form larger cankers than initiated by *Xanthomonas* sp. alone.

Molecular characterisations

Rep-PCR fingerprinting

Rep-PCR using the ERIC and BOXA primers produced nearly identical fingerprints for the Northland and Nelson bacterial isolates (Fig. 3). Fingerprints of the xanthomonads from apricot, peach, poplar, citrus, tomato, and walnut used as controls were easily distinguished from those isolated from olives. Similarity coefficients

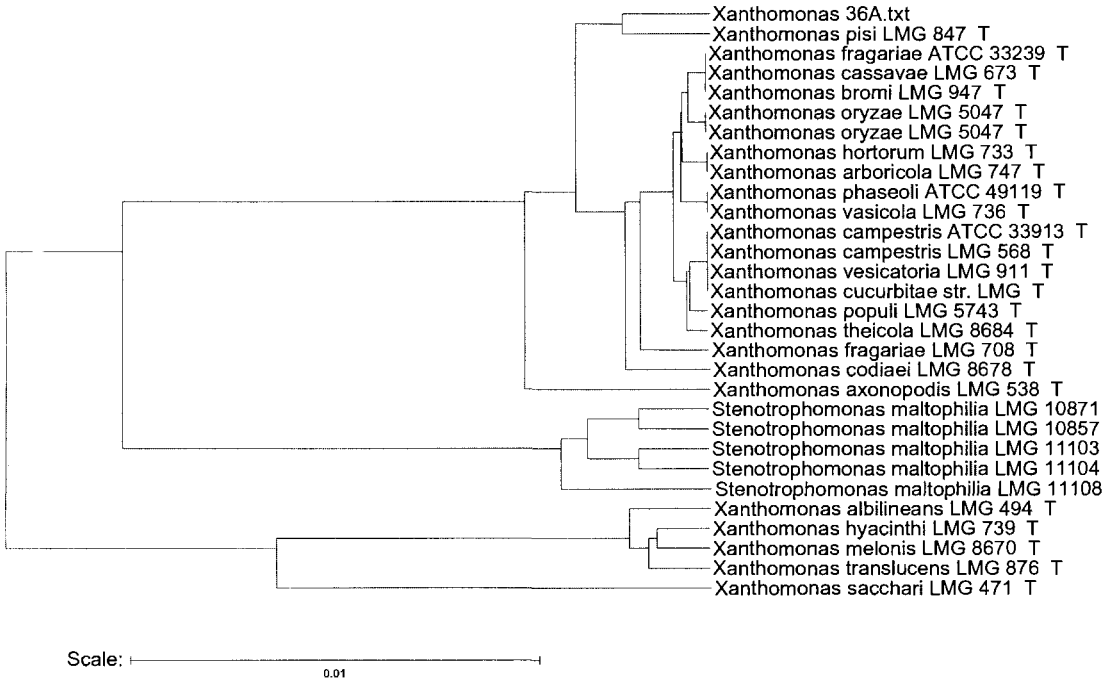

Fig. 4 UPGMA dendrogram estimating the relationships between *Xanthomonas* 36A and amongst species of *Xanthomonas* and *Stenotrophomonas maltophilia*.

calculated between the xanthomonads isolated from olives and those of the control xanthomonads are presented in Table 3.

Sequence determination of the 16S rDNA

A 1409 bp 16S rDNA sequence from the bacterial isolate 36A was determined. The 1409 bp sequence has been deposited in the NCBI GenBank reference number AF335549. The sequence shared 99–99.6% similarity with 16S rDNA of several type species of *Xanthomonas*. Online analysis results from the ribosomal database project indicated that the strains pathogenic to olive in New Zealand belong to the genus *Xanthomonas* and more precisely cluster to the *X. campestris* subgroup. Alignment of the 16S rDNA sequences at *E. coli* positions 80–93 revealed that the olive pathogen shared the same signature nucleotides with 23 species of the *X. campestris* subgroup (data not shown). Hauben et al. (1995) showed that this was the most hypervariable region located in 16S rDNA sequences of the *Xanthomonas* genus and that it was mainly this region that distinguishes the cluster around *X. albilineans* and *Stenotrophomonas maltophilia* from the *X. campestris* core whereas the other groups of *Xanthomonas* and *Stenotrophomonas* genera had different signature nucleotides in this region. A dendrogram revealing the estimated relationships between the *Xanthomonas* sp. isolated from olive and amongst other species of the genus *Xanthomonas* further supported these observations (Fig. 4).

DISCUSSION

This is the first report of a *Xanthomonas* sp. causing a cankerous disease on olive. Small, pale lemon-yellow colonies were consistently isolated from the stem cankers and confirmed as the causal agent of the disease by fulfilling Koch's postulates. On the basis of cultural and biochemical tests, the bacterium isolated from olive fulfilled the description which defines the genus *Xanthomonas* (Dye & Lelliott 1974; Dye et al. 1980). The rDNA sequence suggests that the strains pathogenic to olive belong to the

Xanthomonas genus and that they cluster to the *X. campestris* subgroup. The rDNA sequence from the olive xanthomonad appears to be unique, but unfortunately due to the low level of diversity in the 16S rDNA sequences across members of the *Xanthomonas* genus it is not possible to determine species level relationships.

Initially it was considered that other canker-causing xanthomonads present in New Zealand, may have caused the symptoms observed on olive. *X. c* pv. *populi* and *X. c* pv. *pruni* were two likely candidates present in New Zealand (Young 1976; Haworth & Spiers 1988). However, neither of these pathogens provoked any symptoms on young olive trees in pathogenicity tests. *X. c* pv. *populi* was further distinguished from the olive pathogen on its ability to utilise salicin as a sole carbohydrate source. *X. c* pv. *citri* causes cankers on citrus, but was eradicated from New Zealand in the 1960s. In this study Rep-PCR fingerprinting confirmed that the olive pathogen was not *X. c* pv. *citri*, *X. c* pv. *populi*, *X. c* pv. *pruni*, or *X. c* pv. *juglandis*. Rep-PCR fingerprinting also revealed that the *Xanthomonas* sp. isolated from olive was genetically distinct from the other xanthomonads tested as they shared a genetic homology of only 44–14% (Table 3). There are 21 *X. campestris* pathovars recorded on horticultural crops in New Zealand (Pennycook 1989). However, only three of these cause canker-like symptoms on woody plants and the remaining pathovars cause either leaf spots or twig blights. We consider that it is unlikely that xanthomonads that cause leaf spot or twig blight symptoms on other hosts in New Zealand would also cause this cankerous disease on olive. On this basis we feel that the olive pathogen is unlikely to be one of the xanthomonads that has already been identified in New Zealand affecting either horticultural crops or native plants. To the best of our knowledge there have been no reports of a xanthomonad causing disease on olive worldwide. A possible explanation for the origin of this *Xanthomonas* sp. is that it was introduced on imported olive material. *X. campestris* has been reported to colonise the leaf surface of healthy olives in Israel (Ercolani 1978), suggesting that this organism may exist as an epiphyte, as do many xanthomonads (Hirano & Upper 1983; Graham et al. 1987). The appearance of the stem canker on olive trees in New Zealand may be attributed to favourable conditions for disease expression. It is possible that the *Xanthomonas* sp. reported here is an opportunist pathogen, attacking olives only under certain adverse conditions, which may explain the low incidence of stem canker observed in New Zealand.

Results of pathogenicity tests demonstrated that the *Xanthomonas* sp. isolated from olive trees in New Zealand was pathogenic to young olive trees and reproduced the typical symptoms of stem canker on inoculation. *F. luteum* provoked a necrotic reaction on test plants, but the symptoms differed from those seen in olive groves. Co-inoculation of the two pathogens did not form cankers larger than those initiated by the isolates of *Xanthomonas* sp. alone. The role of *F. luteum* in causing stem cankers is somewhat ambiguous and at this stage it is not clear if it has a role in the primary infections. *F. luteum* is more likely to be associated with tip die-back because of its importance in the development of similar symptoms in other hosts. *F. luteum* is an important pathogen in several New Zealand fruit crops, including avocados (Hartill 1991), grapes (Buchanan & Beever 1987), kiwifruit (Pennycook & Samuels 1985), and other woody plants, particularly those used in shelter belts (Pennycook 1989), and may have spread from these hosts to olives. A number of fungi have been reported to cause symptoms of canker and tip die-back of olive in other countries. Notable among them are *Armillaria* sp., *Cylindrocarpon destructans*, *Phytophthora* sp., *Phoma incompta*, *Diplodia* sp., and *Eutypa lata* (Sanchez Hernandez et al. 1998). There have been reports of other plant pathogenic bacteria on olive, but none of these cause either stem cankers or tip die-back. Significant among them are *Pseudomonas savastanoi* pv. *savastanoi* causing olive knot (Gardan et al. 1992). However, no other pathogenic bacteria were isolated from the diseased olive trees in our study and we have consequently eliminated them as the causal agents.

The convoluted taxonomy of the *Xanthomonas* genus has hampered the specific identification of this particular pathogen on olive. The morphological and biochemical tests used in this study are commonly used for identification as well as taxonomy and nomenclature of the *Xanthomonas* genus. However, these tests cannot reliably distinguish or identify the various species or pathovars in the *Xanthomonas* genus. Molecular classification of the *Xanthomonas* genus has made significant progress, but a simple test has yet to be developed that will provide accurate identification at the pathovar level. Future work to establish the species and pathovar delineation of the *Xanthomonas* sp. isolated from diseased olive trees will require pathogenicity, genomic fingerprinting and DNA hybridisation tests on a large number of

reference strains. We consider that this *Xanthomonas* sp. is a new record on olive causing a stem canker, and cultures of the isolates are deposited with the ICMP and listed as ICMP 13401–13403.

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REFERENCES

Birch, P. R.; Hyman, L. J.; Taylor, R.; Opio, A. F.; Bragard, C.; Toth, I. K. 1997: RAPD PCR-based differentiation of *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans*. *European Journal of Plant Pathology 103*: 809–814.

Buchanan, P. K.; Beever, R. E. 1987: *Botryosphaeria* rot of grapes. *Australian Microbiologist 8*: 209.

Cerny, G. 1976: Method for the distinction of gram-negative from gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology 3*: 223–225.

Dye, D. W. 1962: The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *New Zealand Journal of Science 5*: 393–416.

Dye, D. W.; Bradbury, J. F.; Goto, M.; Hayward, A. C.; Lelliott, R. A.; Schroth, M. N. 1980: International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Review of Plant Pathology 59*: 153–168.

Dye, D. W.; Lelliott, R. A. 1974: The genus *Xanthomonas*. *In*: Buchanan, R. E.; Gibbons, N. E. Bergey's manual of determinative bacteriology. 8th ed. Baltimore, Williams and Wilkins.

Ercolani, G. L. 1978: *Pseudomonas savastanoi* and other bacteria colonising the surface of olive leaves in the field. *Journal of General Microbiology 109*: 245–257.

Gardan, L.; Bollet, C.; Ghorrah, M. Abu.; Grimont, F.; Grimont, P. A. D. 1992: DNA relatedness among the pathovar strains of *Pseudomonas syringae* subsp. *savastanoi* and proposal of *Pseudomonas savastanoi* sp. nov. *International Journal of Systematic Bacteriology 42*: 606–612.

Graham, J. H.; McGuire, R. G. 1987: Survival of *Xanthomonas campestris* pv. *citri* in citrus plant debris and soil. *Plant Disease 71*: 1094–1098.

Gregersen, T. 1978: Rapid method for distinction of gram-negative from gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology 5*: 123–127.

Hartill, W. F. T. 1991: Postharvest diseases of avocado fruits in New Zealand. *New Zealand Journal of Crop and Horticultural Science 19*: 297–304.

Hauben, L.; Vauterin, L.; Swings, J.; Moore, E. R. B. 1997: Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology 47*: 328–335.

Haworth, R. H.; Spiers, A. G. 1988: Stem necrosis of *Populus deltoides* X *P. trichocarpa* in New Zealand caused by *Xanthomonas campestris* pv. *populi*. *European Journal of Forestry Pathology 18*: 200–206.

Hirano, S. S.; Upper, C. D. 1983: Ecology and epidemiology of foliar bacterial plant pathogens. *Annual Review of Phytopathology 21*: 243–269.

King, E. O.; Ward, M. K.; Raney, D. E. 1954: Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine 44*: 301–307.

Louws, F. J.; Fulbright, D. W.; Stephens, C. T.; de Bruijin, F. J. 1994: Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive DNA sequences and PCR. *Applied and Environmental Microbiology 60*: 2286–2295.

Nei, M.; Li, W. H. 1979: Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences 82*: 5078–5082.

Pennycook, S. R. 1989: Plant diseases recorded in New Zealand. Plant Diseases Division, DSIR.

Pennycook, S. R.; Samuels, G. J. 1985: *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon 24*: 445–458.

Rudner, R.; Studamire, B.; Jarvis, E. D. 1994: Determinations of restriction fragment length polymorphism in bacteria using ribosomal RNA genes. *Methods in Enzymology 235*: 184–196.

Sambrook, J.; Fritsch, E. F.; Maniatis, T. 1989: Molecular cloning: laboratory manual. 2nd ed. Cold Spring Harbour Laboratory Press.

Sanchez Hernandez, M. E.; Ruiz Davila, A.; Perez de Algaba, A.; Blanco Lopez, M. A.; Trapero Casas, A. 1998: Occurrence and etiology of death of young olive trees in southern Spain. *European Journal of Plant Pathology 104*: 347–357.

Sands, D.C. 1990: Physiological criteria-determinative tests. *In*: Klement, Z.; Rudolph, K.; Sands, D. C. *ed.* Methods in phyto-bacteriology. Budapest, Akademiai Kiado. Pp. 133–143.

Schaad, N. W.; Stall, R. E. 1988: *Xanthomonas*. *In*: Schaad. W. *ed.* Laboratory guide for identification of plant pathogenic bacteria. St Paul, MN, United States, American Phytopathological Society. Pp. 81–94.

Young, J. M. 1976: *Xanthomonas pruni* in almond in New Zealand. *New Zealand Journal of Agricultural Research 20*: 105–107.