A leaf disc assay for determining resistance of taro to *Phytophthora colocasiae*

J.L. Tyson and R.A. Fullerton

The New Zealand Institute for Plant & Food Research Limited, 120 Mt Albert Road, Auckland 1025, New Zealand

Corresponding author: joy.tyson@plantandfood.co.nz

Abstract *Phytophthora colocasiae* is a destructive fungal pathogen of taro (*Colocasia esculenta*), which causes taro leaf blight. This disease destroyed Samoa's taro export industry and led to local food shortages following introduction of the pathogen in 1993. In support of a Pacific regional taro resistance breeding programme, laboratory studies in New Zealand aimed to develop a leaf disc bioassay for identifying taro genotypes with disease tolerance. A medium has been developed that will retard senescence of the leaf discs for over 14 days. The rate of leaf disc infection and subsequent lesion development varied with incubation temperature, leaf age and taro cultivar. An understanding of these factors and the variation they cause has resulted in a laboratory leaf disc assay for testing resistance of different taro cultivars to taro leaf blight.

Keywords taro leaf blight, *Colocasia esculenta*, temperature, leaf age, cultivar.

INTRODUCTION

In many of the Pacific Island countries, taro (*Colocasia esculenta* var. *esculenta*) is a staple food crop. Almost all parts of taro plants are consumed. The corms are eaten for their high starch content, and the leaves serve as a green vegetable (Miyasaka et al. 2013). Even the petioles and flowers are consumed in some regions of the world (Singh et al. 2012).

Although there are at least 10 major pathogens that affect taro in the Pacific Islands (Kohler et al. 1997), only a few cause serious reduction in growth and production. *Phytophthora colocasiae* is one of the most destructive and causes leaf blight and corm rot of taro. Commonly known as taro leaf blight (TLB), the disease destroyed the Samoan taro export industry and led to localised food shortages following its introduction in 1993 (Anonymous 1993).

At the time of the incursion, the crop was almost genetically uniform, with the cultivar Niue predominating. Niue subsequently proved to be highly susceptible to TLB and was therefore vulnerable to a major disease epidemic (Brunt et al. 2001). Attempts to control the disease using fungicides and management activities such as removal of infected leaves were ineffective because of financial considerations and labour demands. Resistant varieties were seen as the only sustainable solution to the problem, and a breeding programme based on recurrent selection was initiated in Samoa in 1996 using exotic and traditional cultivars as parents (Brunt et al. 2001; Fonoti et al. 2008).

In response to the need for resistant taro varieties, the Australian Agency for International Development (AusAID), in association with

the Secretariat of the Pacific Community (SPC) established a taro breeding programme within the Taro Genetic Resources: Conservation and Utilisation project (TaroGen), to develop resistant varieties for use in Papua New Guinea and Samoa. Plant pathology inputs to the programme were supported by the New Zealand Overseas Development Assistance programme.

In order to screen the new cultivars for resistance, an easy and replicable method of screening was needed. Methods to assess resistance of taro cultivars and breeding progeny in the field were evaluated in Samoa and Papua New Guinea (PNG) over 1999–2002 (J.L. Tyson, unpublished data). However, results of field evaluations were variable, with individual cultivars often responding quite differently in successive tests.

Initial laboratory experiments to determine the cause of this variability showed that the pathogen is strongly influenced by temperature. Field observations were similar; if cool, wet conditions prevailed after inoculation, symptom development was rapid. During hot, dry conditions, lesions developed slowly, and in some the pathogen died out and the lesions failed to expand further. The extreme effect of environmental conditions on symptom development makes field assessments of resistance unreliable.

The research reported here aimed to develop laboratory screening techniques for taro leaf blight, and largely took place between 1999 and 2003.

MATERIALS AND METHODS

A collection of isolates of *Phytophthora colocasiae* was established; details are given in Tyson & Fullerton (2007). Isolates were maintained on V8 juice agar (100 ml V8 juice, 2 g CaCO₃, 15 g agar, 900 ml distilled water) (Hine & Aragaki 1963).

Anti-senescence medium for taro leaf discs

Leaf discs of taro (60 mm diameter, eight discs per substrate) were placed into Petri dishes (9 mm diameter) containing either water, wet filter paper, water agar (15 g/litre), water agar (7.5 g/litre), water agar (7.5 g/litre) + 150 µg/litre benzimidazole, or water agar (7.5 g/litre) + 150 µg/litre benzimidazole + 1.25 ml/litre Triton X. Leaf discs were assessed after 3, 6, 8, 10 and

14 days, and the percentage of green leaf area remaining was recorded at each assessment.

Effects of temperature on *Phytophthora colocasiae*

Petri dishes of potato dextrose agar (PDA) were inoculated with 3 mm mycelial plugs taken from 2-day-old cultures of *P. colocasiae* (isolate Pc35, five plates per temperature). The PDA plates were incubated at 15, 20, 25, 30 or 35°C and mycelial growth assessed after 3, 4 and 5 days. The experiment was repeated once.

Taro leaf discs (60 mm diameter) taken from the second fully unfurled leaf of taro cultivar Niue were kept on water agar (7.5 g/litre) + 150 µg/litre benzimidazole + 1.25 ml/litre Triton X. Leaf discs were inoculated with 6 mm mycelial plugs taken from 2-day-old cultures of *P. colocasiae* (isolate Pc35) and held at 15, 20, 25, 30 and 35°C (five discs per temperature). Symptoms were assessed after 4 days. The experiment was repeated once.

Effect of leaf age

Taro leaf discs (60 mm diameter) taken from the first, second or third fully unfurled leaves of taro cultivars Niue, Pastora or P7 were placed on water agar (7.5 g/litre) + 150 µg/litre benzimidazole + 1.25 ml/litre Triton X. Leaf discs were inoculated with 6 mm mycelial plugs taken from 2-day-old cultures of *P. colocasiae* (isolate Pc35) and held at 25°C (10 discs per cultivar). Symptoms were assessed after 4 days. The experiment was repeated once.

Statistical analysis

Results were analysed using Analysis of Variance (ANOVA). Differences between individual treatments were determined using Fisher's Least Significant Difference (LSD, $P=0.05$). All analyses were conducted using Minitab version 16.

RESULTS

Anti-senescence medium for taro leaf discs

Taro leaf discs had the most rapid rate of senescence on wet filter paper and water agar (15 g/litre). The discs remained green for the full 14 days of the experiment (Table 1) on water agar (7.5 g/litre) amended with benzimidazole and Triton X.

Effects of temperature on *Phytophthora colocasiae*

Phytophthora colocasiae grew on PDA over the temperature range 15–30°C. Because the growth rates at each temperature were very similar in both experiments, the means were combined for presentation (Table 2). Colony growth was completely inhibited at 35°C. In the leaf disc assays, temperature had a statistically significant effect on lesion size and percent successful infections ($P=0.05$). At 15°C the rate of infection

of leaf discs was very low (Table 3). The rate of development of lesions on leaf discs was greatest at 25 and 30°C, but suppressed at 35°C.

Effect of leaf age

The most consistent results and best discrimination between taro genotypes was obtained when discs were taken from the youngest fully expanded leaves of plants (Figure 1), although lesion development was generally greater on leaf discs taken from second or third leaves.

Table 1 Mean proportions (%) of green area for taro leaf discs held in Petri dishes containing different media for up to 14 days. Means are of eight leaf discs.

Medium	Day 3	Day 6	Day 8	Day 10	Day 14
Water	98.1	90.6	70.6	50.0	20.6 b ¹
Wet filter paper	94.4	81.9	66.3	36.3	0.6 c
Water agar (15 g/litre)	94.4	86.3	62.5	30.0	0.6 c
Water agar (7.5 g/litre)	98.1	89.4	71.9	43.1	8.1 bc
Water agar (7.5 g/litre)+150 µg/litre benzimidazole	96.3	67.5	52.5	30.0	18.8 b
Water agar (7.5 g/litre)+150 µg/litre benzimidazole+1.25 ml/litre Triton X	100.0	98.8	98.8	98.8	98.8 a

¹Day 14 means with the same letter are not significantly different (Fisher's LSD, $P<0.05$)

Table 2 Mean colony diameters (mm) of *Phytophthora colocasiae* grown on potato dextrose agar for 3, 4 or 5 days at different temperatures. Values are combined means ($n=10$) from two experiments.

Day	15°C	20°C	25°C	30°C	35°C
3	7.0	18.0	21.0	19.5	0.0
4	9.0	22.5	26.5	23.5	0.0
5	10.5	27.0	32.0	28.0	0.0

Table 3 Lesion diameters (mm) and proportions (%) of successful infections on leaf discs of taro cultivar Niue inoculated with *Phytophthora colocasiae* and maintained for 4 days at different temperatures. Values are the mean from two experiments.

Temperature (°C)	Experiment 1		Experiment 2	
	Mean lesion diameter ¹	% successful infections	Mean lesion diameter ¹	% successful infections
15	7.0 b ²	20	0.0 d ²	0
20	16.6 a	100	18.4 c	100
25	27.8 a	100	27.6 b	100
30	35.3 a	60	39.4 a	100
35	0.0 b	0	0.0 d	0

¹Mean lesion diameter of successful infections.

²Means with the same letter are not significantly different (Fisher's LSD, $P<0.05$).

Figure 1 Mean lesion diameters on leaf discs of three ages from three different taro cultivars, 4 days after inoculation with *Phytophthora colocasiae* in an *in vitro* assay. Means within a group with the same letter are not significantly different (Fisher's LSD, $P < 0.05$).

DISCUSSION

Resistance screening of taro cultivars in the field is strongly influenced by environmental factors. This project aimed to develop a laboratory screening technique to assess cultivars for susceptibility to taro leaf blight.

Taro leaf tissue undergoes rapid senescence once removed from plants. Benzimidazole has been previously used in detached leaf assays to inhibit leaf senescence (Person et al. 1957; Colin et al. 1990). In the present study it was found that addition of the surfactant Triton X to the media was essential to allow contact between the leaf discs and the medium, and hence the uptake of benzimidazole. This is because taro leaves are extremely hydrophobic. The addition of both compounds to water agar successfully retarded senescence of the leaf discs for over 14 days, a period adequate to evaluate genotype *in vitro* responses to inoculations.

Phytophthora colocasiae grew on agar media and leaf discs over the temperature range 15–30°C. The most suitable temperature at which to incubate leaf disc assays was found to be 25°C. At this temperature the lesions grew quickly and the greatest number of successful infections

occurred. It was also possible to differentiate between the rate of lesion development on different taro cultivars at this temperature (J.L. Tyson, unpublished data).

This research has shown that *P. colocasiae* is strongly influenced by temperature. Optimum growth occurred within the range 25–30°C, and at 35°C growth ceased. Temperatures in the field in Samoa and Papua New Guinea exceed this critical temperature. This strong temperature effect partly explains field experience, where symptom development was rapid when cool, wet conditions prevailed after inoculation. If conditions were hot and dry, lesion development was slow, and in some cases the pathogen died and the lesion failed to expand further. This effect of temperature on symptom development makes field assessments of resistance unreliable.

Previous research has shown that the rate of disease development observed on taro in the field differs with leaf age (J.L. Tyson, unpublished data). This has also been the experience of Brooks (2008), who found that older leaves were more susceptible to TLB than young leaves. For consistent results it is therefore necessary to standardise the age of the leaves selected for leaf disc assays.

The present studies have shown that incubation temperature, leaf age and taro genotype can all influence the incidence of infection and rate of lesion development on taro leaf discs. Standardisation of these various factors has enabled the development of a leaf disc assay protocol for evaluating different genotypes for TLB resistance.

The leaf disc assay based on these results uses 10 discs (60 mm diameter) from the youngest fully expanded taro leaf. Discs should be placed on water agar (7.5 g/litre) amended with 150 µg/litre benzimidazole and 1.2 ml/litre Triton X. Each disc should be inoculated in the centre with a 6 mm plug taken from the margin of an actively growing colony of *P. colocasiae*. Inoculated leaf discs should be incubated for 4 days at 25°C in the dark, then lesion diameters measured and % successful infections recorded. Known resistant and susceptible taro genotypes should be included in each test as standards.

Subsequent to these studies, Iramu et al. (2004) evaluated the leaf disc assay against other methods to screen taro genotypes for TLB resistance. They concluded that the leaf disc assay was the most precise and reliable method for screening small numbers of genotypes.

ACKNOWLEDGEMENTS

This research was funded by the New Zealand Ministry of Foreign Affairs and Trade as part of the AusAID TaroGen project.

REFERENCES

- Anonymous 1993. Taro blight in Western and American Samoa. South Pacific Commission. <http://www.spc.int/paps/PestAlerts/AgAlertNo10.pdf> (accessed 1 Nov 2014).
- Brooks FE 2008. Detached-leaf bioassay for evaluating taro resistance to *Phytophthora colocasiae*. Plant Disease 92: 126-131.
- Brunt J, Hunter D, Delp C 2001. A bibliography of taro leaf blight: AusAID/SPC taro genetic resources: conservation and utilisation. Secretariat of the Pacific Community, Noumea, New Caledonia. 96 pp.
- Colin J, Bragard C, Maraite H 1990. A detached leaf inoculation method for pathogenicity testing of *Xanthomonas campestris* on cereals. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 55: 1125-1131.
- Fonoti P, Tofinga MP, Hunter DG 2008. Screening a cycle 1 breeding population of taro (*Colocasia esculenta* (L.)) for resistance to taro leaf blight in Samoa. Research Journal of Biological Sciences 3: 888-891.
- Hine RB, Aragaki M 1963. Pathogenicity, vitamin nutrition and cultural characteristics of isolates of *Phytophthora parasitica* from carnation and other hosts in Hawaii. Phytopathology 53: 1194-1197.
- Iramu ET, Akanda S, Wagih ME, Singh D, Fullerton RA 2004. Evaluation of methods for screening taro (*Colocasia esculenta*) genotypes for resistance to leaf blight caused by *Phytophthora colocasiae*. Papua New Guinea Journal of Agriculture, Forestry and Fisheries 47: 37-44.
- Kohler F, Pellegrin F, Jackson G, McKenzie EHC 1997. Diseases of cultivated crops in Pacific Island countries. South Pacific Commission, Noumea, New Caledonia. 187 pp.
- Miyasaka SC, Lamour K, Shintaku M, Shrestha S, Uchida J 2013. Taro leaf blight caused by *Phytophthora colocasiae*. In: Lamour K ed. *Phytophthora: a global perspective*. CABI Plant Protection Series. CAB International. Pp. 104-112.
- Person C, Samborski DJ, Forsyth FR 1957. Effect of benzimidazole on detached wheat leaves. Nature 180: 1294-1295.
- Singh D, Jackson G, Hunter D, Fullerton RA, Lebot V, Taylor M, Iosefa T, Okpul T, Tyson JL 2012. Taro leaf blight - a threat to food security. Agriculture 2012: 182-203.
- Tyson JL, Fullerton RA 2007. Mating types of *Phytophthora colocasiae* from the Pacific region, India and South-east Asia. Australasian Plant Disease Notes 2: 111-112.