Sweetpotato cultivar susceptibility to infection by *Ceratocystis fimbriata*

S.L. Lewthwaite[1], P.J. Wright[1] and C.M. Triggs[2]

[1]*The New Zealand Institute for Plant & Food Research Limited, Cronin Road, RD1, Pukekohe, New Zealand*
[2]*Department of Statistics, University of Auckland, Private Bag 92019, Auckland, New Zealand*
Corresponding author: Steve.Lewthwaite@plantandfood.co.nz

Abstract The fungus *Ceratocystis fimbriata* causes a disease of the sweetpotato (*Ipomoea batatas*) plant commonly known as black rot. This study evaluated sweetpotato cultivar susceptibility to *C. fimbriata* infection. During crop production, infection of sweetpotato storage roots may take place by transmission from contaminated transplants, but generally the pathogen is introduced directly through openings in the periderm. These openings may take the form of damaged secondary lateral roots, lenticels or wounds. In a laboratory-based bioassay, storage roots were punctured then point-inoculated with the pathogen. Following incubation under warm humid conditions, the dimensions of black rot lesions were compared. The predominant New Zealand cultivar 'Owairaka Red' was demonstrably less susceptible to *C. fimbriata* than the Japanese cultivar 'Beniazuma', but significantly more susceptible than 'Beauregard' from the United States of America ($P<0.001$). The rate of increase in lesion diameter for the most susceptible cultivar assessed was almost twice that of the least susceptible.

Keywords kumara, fungus, disease, resistance, black rot.

INTRODUCTION

The pathogen *Ceratocystis fimbriata* Ell. & Halst. causes a fungal disease of the sweetpotato (*Ipomoea batatas* (L.) Lam.) or kumara plant commonly known as black rot. This fungus was initially identified within North American sweetpotato crops in 1890 (Halsted 1890), with the first New Zealand recording in 1907 (Kirk 1907). The disease was recognised as a significant problem for New Zealand sweetpotato growers from 1947 when it was recorded at Kaitaia, and then over subsequent years was recognised within other sweetpotato producing regions of Northland, Auckland and the East Coast (Slade 1960). Sweetpotato production entered a period of decline following these black rot outbreaks, which for some growers caused severe and occasionally complete, crop loss. After the local industry adopted the strict crop hygiene protocols introduced by the New Zealand Department of Agriculture (Coleman 1962) sweetpotato production increased, with black rot ceasing to be a problem and being rarely observed thereafter (Quinn 1968). However, the New Zealand sweetpotato industry has continued to expand over the decades, with changes to personnel and crop management

systems. In recent years the black rot disease has been found more frequently, so further work is required to investigate contributing factors and the integration of control measures.

The black rot fungus has many strains, each with a different host plant range. The sweetpotato strain appears to be specific to the Convolvulaceae family. There is some evidence that Convolvulaceous weed species also host *C. fimbriata*, producing typical disease lesions (Clark & Watson 1983). In sweetpotato, the black rot fungus only directly affects the underground portions of the plant and roots in storage; primary symptoms are not seen above ground in the growing crop (Slade 1960). In plants, small black lesions form, enlarge and merge on buried portions of the stem. Sometimes the underground stems may become completely girdled with lesions. The leaves of infected plants in propagation beds may turn yellow, wilt and shed, while plant growth may be stunted or show signs of collapse. Severe infection pressure may cause the death of new sprouts before they can emerge from the soil (Coleman 1972).

In sweetpotato storage roots, black rot infection generally takes place through openings in the skin such as damaged lateral roots, lenticels or wounds. Infected storage roots initially show circular brown lesions that are slightly depressed. The lesions become grey-black in colour, but when wet appear green-black (Clark & Moyer 1988). Lesions are initially small, but increase in size with distinct edges until they merge. The infected tissue is firm and dry, with the periderm usually remaining intact. Lesions do not generally penetrate below the vascular ring unless a secondary infection takes place (Clark & Moyer 1988). A cluster of perithecia often forms and produces ascospores at the centre of the lesion. Infected roots may produce a strong fruity smell, which is thought to attract insects that help spread the sticky ascospores. Although badly diseased roots are often noticed and discarded at harvest, those without obvious visible symptoms may be retained and subsequently produce a general infection during storage.

Phytoalexins are naturally induced antibiotics that contribute to a plant's self defence. The first phytoalexin isolated and identified in the plant kingdom was obtained from diseased sweetpotato roots and named ipomeamarone (Hiura 1943). In sweetpotato, living plant tissue may respond to sustained biological, chemical or physical attack by producing phytoalexins (Woolfe 1992). While phytoalexins are not found in healthy roots they are induced by persistent stressors; amongst sweetpotato pathogens *C. fimbriata* is considered a high inducer of phytoalexins (Clark et al. 1981). Sweetpotato cultivars may differ in their level of phytoalexin induction (Martin et al. 1978). Phytoalexins make sweetpotato roots toxic and unpalatable due to a bitter taste. While there is a well established history of cattle deaths as a direct consequence of consuming diseased sweetpotato roots, there have been no reports of human poisoning (Woolfe 1992).

The fungus *C. fimbriata* is readily spread through direct contact with infected equipment and plant matter, but is also dispersed by the movement of air, water, animals and insects. It may cause severe economic losses within production beds, the field and during storage. This study developed and evaluated a bioassay procedure to assess relative cultivar susceptibility as a contributive factor in disease development.

MATERIALS AND METHODS

A sample of *C. fimbriata*, isolated from a sweetpotato storage root, was deposited in the International Collection of Micro-organisms from Plants (ICMP) as ICMP number 13575 (Young & Fletcher 1999). Sterile Petri dishes containing potato dextrose agar were inoculated with pure cultures of this fungal specimen and incubated at 27°C for 15 days. Following incubation 12 colonies were carefully scraped from the agar surface, filtered through two layers of cheese cloth and mixed with distilled water to a total volume of 40 ml. The resulting suspension, with a concentration of 1.67×10^7 conidia/ml as determined by hemocytometer, was used to inoculate sweetpotato storage roots.

Sweetpotato storage roots harvested at the Pukekohe Research Centre were stored in 2-ply multi-walled paper bags at ambient temperature

for 3 months. Eight diverse sweetpotato cultivars were selected for the experiment, namely: 'Owairaka Red' (Lewthwaite 1998), the predominant New Zealand cultivar with red-purple skin and cream flesh; 'Beauregard' (Rolston et al. 1987), the dominant USA cultivar with orange skin and flesh, currently the second most popular cultivar in New Zealand; 'Toka Toka Gold' (Lewthwaite 1998), a New Zealand cultivar with cream skin and cream-orange flesh, currently the third most prevalent New Zealand sweetpotato cultivar; 'Beniazuma' (Shiga et al. 1985), a red-skinned cream-fleshed cultivar popular in Japan; 'Northland Rose' (Broadhurst et al. 1997), formerly breeding line 93N9/2, a cultivar selected in 1993 from a segregating seed population supplied by the Asian Vegetable Research and Development Centre (AVRDC), Taiwan; 'Radical' (Philpott et al. 2003), formerly breeding line 99N1/222, a purple skinned and fleshed cultivar selected in 1999 from a segregating seed population supplied by the Kyushu National Agricultural Experiment Station (KNAES), Japan; 'Purple Star', formerly breeding line C42 (file number 02N4/2), a red-purple skinned cultivar with mottled purple-cream flesh, originally selected in 2002 from a segregating seed population supplied by the International Potato Centre (CIP), Peru; and 'S1819' (file number 04N1819), a clone with red skin, and flesh mottled with both orange and purple, selected in 2004 from a segregating seed population supplied by the International Potato Centre (CIP), Peru.

Twenty healthy storage roots, ranging in weight from 200 to 300 g, were selected for each cultivar. The roots were hand-washed and their surfaces air-dried, before being placed in 10 high humidity storage chambers. To maintain humidity, the base of each chamber was lined with corrugated cardboard moistened with distilled water. The experiment was arranged as a randomised complete block design with chambers as blocks. Two roots of each cultivar were placed in each of the 10 chambers, thus each cultivar was replicated 20 times.

At inoculation, each root was pierced to a depth of 4.5 mm with an ethanol/flame sterilised 2 mm diameter steel rod and the resulting cavity filled with the *C. fimbriata* suspension. Every root was inoculated at two sites, separated by approximately 10 cm. The chambers were incubated at 20°C and the roots were assessed for lesion size at 13 and 19 days from inoculation. The diameter of each lesion was measured twice, along two perpendicular axes, with the mean diameter length calculated for each root. The ANOVA and linear regression procedures of the software GENSTAT® were used for statistical analysis.

RESULTS AND DISCUSSION

All of the inoculation points developed well defined lesions. Therefore these results (Table 1) are consistent with previous work, in that while sweetpotato cultivars varied in their degree of black rot susceptibility, none appeared immune (Cheo 1953; Nielsen & Yen 1966). The cultivars evaluated here showed a significant range of responses (Table 1) when assessed at either 13 or 19 days from inoculation ($P<0.001$). Accurate measurement of lesion size was more easily made when assessment was delayed, but prolonged incubation risked the confounding factors of coalescing lesions, secondary infection, lenticel enlargement and sprouting. Repeated measurement of lesion diameter during incubation provided an opportunity to determine the pattern (Figure 1) and rate (Table 1) of lesion growth. Based on the three data points available for each lesion (2 mm diameter initial puncture wound, lesion diameter at 13 and 19 days), lesion development was well fitted by a linear growth model (Figure 1). While lesions appeared to grow linearly over the 19 day duration of the experiment, further assessment was impractical due to confounding factors. The linear model illustrated the diverging nature of cultivar responses, apparent with prolonged incubation.

The cultivar 'Beauregard', which is marketed internationally and widely consumed, is considered relatively tolerant to fungal infection. In this bioassay, the black rot susceptibility of clone 'S1819', a selection being considered for commercial release, did not differ from that of 'Beauregard' ($P<0.001$). A

newly released cultivar, 'Purple Star', showed a significantly lower susceptibility to black rot than 'Beauregard' (P<0.001). The remaining cultivars showed a range of responses, all of which were significantly more prone to black rot infection than 'Beauregard'. Of this latter group, the predominant New Zealand cultivar 'Owairaka Red' was the least susceptible, while the novel purple-fleshed cultivar 'Radical' was most susceptible (Table 1). The rate of increase in lesion diameter for the most susceptible cultivar, 'Radical', was almost twice that of the least susceptible, 'Purple Star'.

Black rot disease can be limited to economically insignificant levels by an integrated approach to plant hygiene that encompasses the entire cropping cycle. The availability of cultivars that are less susceptible to black rot provides an

Table 1 Diameters (mm) and growth rates (mm/day) of lesions caused by the fungus *Ceratocystis fimbriata* Ell. & Halst. on storage roots of eight sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars. Roots were point-inoculated, incubated at 20°C with high relative humidity, then assessed 13 days (First record) and 19 days (Second record) later.

Cultivar	First record	Second record	Growth rate
Purple Star	10.2	13.2	0.60
Beauregard	10.9	14.9	0.68
S1819	11.1	14.9	0.69
Owairaka Red	12.9	17.4	0.82
Toka Toka Gold	13.9	20.1	0.94
Beniazuma	14.5	20.5	0.97
Northland Rose	15.5	20.2	0.98
Radical	16.7	24.5	1.17
LSD (P=0.05; df=143)	0.96	1.6	0.050
P-value	<0.001	<0.001	<0.001

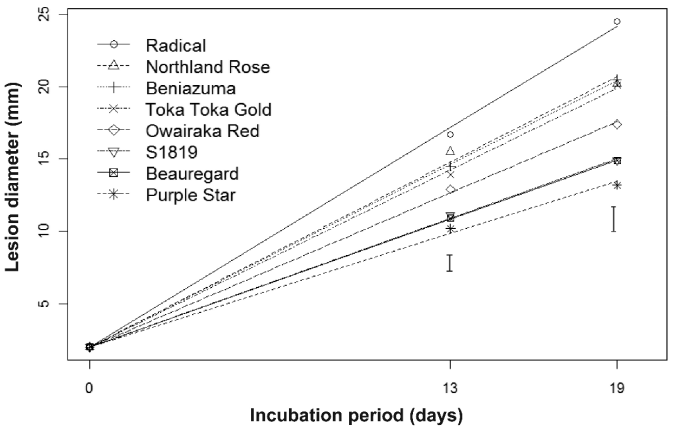

Figure 1 Disease lesion expansion rates for the storage roots of eight sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars, following point-inoculation with the fungus *Ceratocystis fimbriata* Ell. & Halst. Lines represent fitted responses and points correspond to observed mean lesion diameters, for roots incubated at 20°C with high relative humidity. Least significant differences (P=0.05) of 0.96 and 1.6 mm are indicated, for comparing responses following 13 and 19 days of incubation respectively.

opportunity to lower the potential for infection at a number of stages. Only roots that are judged disease-free, by visual inspection of the roots themselves but also based on knowledge of their derivation, should be used as seed in the propagation bed. Any equipment, materials or structures that come in contact with the seed roots and sprouts during propagation must be rendered pathogen-free by disinfection. Fungicides may be applied to the roots within a propagation bed, to destroy spores or small indiscernible infections. Bedded roots are covered with free draining soil then maintained in plastic cloches, raising the temperature and relative humidity to promote rapid sprout growth. At transplanting the sprouts should be cut from the bed, above soil level. Pulled plants, with their attached roots, are more likely to transfer infection from the seed roots. Crop rotation is an important tool for lowering potential infection levels, but its use within the New Zealand production system is limited due to few economically viable alternative crops. At harvest the storage roots should be cured to heal wounds caused by vine removal, secondary root removal, abrasion and impact. Over-curing, due to prolonged storage at high temperature and high relative humidity, results in the development of sprouts that are prone to breakage, and should be avoided. Once the roots are cured, it is important to minimise any further movement that might produce new wounds, prior to washing and shipping to the market. It is essential that diseased roots do not reach the market to avoid consumer resistance and possible exposure to phytoalexins.

Based on this bioassay, assessment of lesion growth rate (Table 1) appears a useful measure of black rot susceptibility, providing an index that is independent of incubation period and broadly derived from multiple measurements. Inclusion of a widely grown cultivar, such as 'Beauregard', provides the bioassay with a common check factor that accounts for other variables such as inoculum virulence and incubation conditions. However, since sweetpotato root susceptibility to some fungal diseases may change during storage (Clark et al. 2009), further work is needed to assess how root production systems and prolonged storage affect cultivar susceptibility and phytoalexin response (Clark et al. 1981).

ACKNOWLEDGEMENTS

This work was supported by the New Zealand Foundation for Research, Science and Technology. We would like to acknowledge the input of Tahuri Whenua and M. Jeram for technical support.

REFERENCES

Broadhurst PG, Lewthwaite SL, Triggs CM 1997. Field evaluation of sweetpotato cultivars for resistance to Sclerotinia rot. Proceedings of the 50th New Zealand Plant Protection Conference: 89–92.

Cheo PC 1953. Varietal differences in susceptibility of sweet potato to black rot fungus. Phytopathology 43: 78–81.

Clark CA, Holmes GJ, Ferrin DM 2009. Major fungal and bacterial diseases. In: Loebenstein G, Thottappily G ed. The sweetpotato. Springer, Heidelberg, Germany. Pp. 81–103.

Clark CA, Lawrence A, Martin FA 1981. Accumulation of furanoterpenoids in sweet potato tissue following inoculation with different pathogens. Phytopathology 71: 708–711.

Clark CA, Moyer JW 1988. Compendium of sweet potato diseases. American Phytopathological Society Press, Minnesota, USA. 74 p.

Clark CA, Watson B 1983. Susceptibility of weed species of Convolvulaceae to root-infecting pathogens of sweet potato. Plant Disease 67: 907–909.

Coleman BP 1962. Vegetable production: Hygienic methods essential for propagating kumara plants. New Zealand Journal of Agriculture 105: 431, 433, 435.

Coleman BP 1972. Kumara growing. New Zealand Department of Agriculture, Bulletin 294. Government printer, AR Shearer, Wellington, New Zealand. 44 p.

Halsted BD 1890. Some fungous diseases of the sweet potato. The black rot. New Jersey Agriculture Experiment Station Bulletin 76: 7–14.

Hiura M 1943. Studies on storage and rot of sweet potatoes (2). Report of the Gifu Agricultural College 50: 1–5.

Kirk TW 1907. Divisions of Biology, Horticulture, and Publications. Report of TW Kirk, Biologist, Chief of Divisions. New Zealand Department of Agriculture. Annual Report 15: 135–258.

Lewthwaite SL 1998. Commercial sweetpotato production in New Zealand: foundations for the future. In: LaBonte DR, Yamashita M, Mochida H ed. Proceedings of the International Workshop on Sweetpotato Production Systems Toward the 21st Century. Kyushu National Agricultural Experiment Station, Japan. Pp. 33–50.

Martin WJ, Hasling VC, Catalano EA, Dupuy HP 1978. Effect of sweet potato cultivars and pathogens on ipomeamarone content of diseased tissue. Phytopathology 68: 863–865.

Nielsen LW, Yen DE 1966. Resistance in sweetpotato to the scurf and black rot pathogens. New Zealand Journal of Agricultural Research 9: 1032–1041.

Philpott M, Gould KS, Markham KR, Lewthwaite SL, Ferguson LR 2003. Enhanced coloration reveals high antioxidant potential in new sweetpotato cultivars. Journal of the Science of Food and Agriculture 83: 1076–1082.

Quinn J 1968. Profitable kumara growing on flats in Northland. New Zealand Journal of Agriculture 116: 17–21.

Rolston LH, Clarke CA, Cannon JM, Randle WM, Riley EG, Wilson PW, Robbins ML 1987. 'Beauregard' sweet potato. HortScience 22: 1338–1339.

Shiga T, Sakamoto S, Ando T, Ishikawa H, Kato S, Takemata T, Umehara M 1985. On a new sweet potato cultivar 'Beniazuma'. Bulletin of the National Agriculture Research Center 3: 73–84.

Slade DA 1960. Black rot an important disease of kumaras. New Zealand Journal of Agriculture 100: 375, 377–378.

Woolfe JA 1992. Sweet potato: an untapped food resource. Cambridge University Press. 643 p.

Young JM, Fletcher MJ 1999. International Collection of Micro-Organisms from Plants: catalogue accessions 12990-13771: 3rd edition supplement. Landcare Research, Lincoln, New Zealand. 45 p.