

A Technique for Detecting Red Stele (*Phytophthora fragariae*) Infection of Strawberry Stocks Before Planting

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ABSTRACT

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The red stele pathogen, *Phytophthora fragariae* Hickman, was detected by a "root-tip bait" test in certified commercial stock strawberry plants, supposedly free of disease. Susceptible bait plants were grown in a mixture of compost and root tips cut from runner plants. When root-tip samples were prepared from a combination of 1:99 infected/uninfected runner plants, the test gave positive results in all 10 trials.

Red stele is a serious root disease of strawberries caused by *Phytophthora fragariae* Hickman. Although the pathogen was widely believed to be soilborne, early workers (1,5) recognized that it was often spread by planting runners dug from apparently healthy propagation beds.

Legislative measures to minimize such accidental spread have been in force in the United Kingdom for nearly 30 years. Thus in Scotland it is an offense to sell runner plants that have not been inspected and certified by the Department of Agriculture and Fisheries for Scotland, if produced locally, or, if produced in England or Wales, have not been certified for sale in Scotland by the Ministry of Agriculture, Fisheries and Food. Although uncertified stocks may be offered for sale in England and Wales, this practice is not recommended. To minimize the spread of the disease in these two countries, the Ministry must be notified of all new outbreaks of red stele, and an order is issued forbidding sale of strawberry plants from that land.

Despite these measures, outbreaks of the disease occur on land with no previous history of strawberry growing even when planted with certified stocks. Thus, either the fungus is present in the soil before planting or it escapes detection during inspection of propagation beds and is disseminated in the planting stocks.

Government inspectors initially seek only aerial symptoms, eg, reduction in vigor, wilting, and leaf coloration. If these symptoms are seen, the roots will be examined for red stele and the presence of oospores. Unfortunately, runners taken from infected but symptomless resistant cultivars can transmit the disease when transplanted adjacent to susceptible cultivars in clean soil (4). It has also been

suggested that susceptible cultivars may similarly transmit infection if taken from infected propagation beds where soil conditions were not conducive to symptom expression (6). Bait plants have been used to detect the pathogen both in soil samples (2,7) and directly in the field (3), and it is therefore possible to check the health status of potential planting sites.

This article describes a development of the root-tip bait test that has been used successfully to check the disease status of planting stocks and that could be used routinely as an aid in certification procedures.

MATERIALS AND METHODS

Three sources of planting material were tested. Commercial stocks of cultivars Cambridge Favourite (CF-2) and Cambridge Vigour (CV), both of English Special Stock grade, were delivered in clean paper sacks containing 1,000 plants in bundles of 25. A second stock of Cambridge Favourite (CF-1) was dug from propagation beds at the Scottish Horticultural Research Institute (SHRI). Root tips, 2.0–2.5 cm long, were taken in early April 1978 from every 20th bundle of the commercial stock plants and from all plants of the SHRI stock. The tips were collected in clean polythene bags and held at 5 C for 1 or 2 days before use.

University of California (UC) soil-less compost (1:3 sand/peat, plus fertilizers, trace elements, and lime; final pH, about 5.5) was mixed 3:1 by volume with root tips. The mixtures were put into 15-cm-diameter plastic plant pots, and each was then planted with 10 plants, about 7.5–10.0 cm tall, of the Alpine strawberry cultivar Baron Solemacher, grown from seed. The seedlings had been transplanted into trays of UC compost 2–3 wk after sowing and grown for 4–6 wk in a growth cabinet at 20 C under continuous light (8,000 lux). All crowns but one and all old leaves on each plant were removed before use.

The pots were placed on a bench

designed to collect all pot drainage water to minimize local contamination. Glasshouse temperature was maintained at 15 C. Plants were watered by mist irrigation for 15 min every 6 hr. After 5 wk the roots of each bait plant were examined for the typical red steles and oospores. Control plants grown in compost alone were placed at random among test plants to detect the possibility of cross-contamination.

Further tests were done on surplus planting material of CF-2 retained by the grower. This material was rotted extensively by the time that the results of the first experiment were available. Red steles could not be seen, and oospores were not detected in the roots. Close examination of them suggested that although the CF-2 plants had been supplied as one stock, they had probably come from several sites. They were divided into four groups according to the soil adhering to their roots (red, yellow, black/peaty, and uncertain), and decayed root-tip samples were taken from each group. Because of the limited sample size, the ratio of root tip to compost was changed to 1:15. All other conditions were the same as before.

In an experiment to check the sensitivity of the test, runner plants of Cambridge Favourite were dug from a 3-yr-old plantation at SHRI that showed no evidence of the disease. Plants were further checked by potting them individually in UC compost in 10-cm-diameter plastic pots and keeping them well watered at 15 C in the glasshouse for 3 wk. They quickly developed extensive root systems that remained free of red stele symptoms, confirming that the field site was free from infection. They were then transferred to the drainage benches and inoculated on 3 successive days by pipetting 10 ml of zoospore suspension containing about 3,000 zoospores/ml over the surface of the compost (8). When washed free of compost 2–3 wk later, their root systems had extensive red steles with abundant typical oospores.

Plants were next dug from the original plantation, and the fresh weight of their root tips ranged from 28 to 72 g per lot of 100 sampled plants; the average for 10 lots was 45.3 g, indicating an average of about 0.5 g of root tips per plant. Using this per plant approximation, infected root tips were incorporated at fresh weights equivalent to 1, 5, and 10 infected plants with root tips from 99, 95, and 90 newly dug plants, respectively. Ten sets of

samples were prepared for each of the three levels, together with the same number of check samples, each consisting of tips from 100 newly dug plants. Each sample was mixed with compost, potted in a 12.5-cm-diameter plastic pot, and planted with five bait plants. Smaller pots were used in this experiment to match the quantity of plants available for testing and because pots of this size would be more suitable for the sample sizes likely to be taken in commercial practice. As before, the control pots contained compost alone. The baiting technique and the temperature and watering regimes were the same as used in the first experiment.

RESULTS

Of the two commercial stocks, some plants of CF-2 must have been infected before delivery to the grower (Table 1). Of 30 runners dug at random from the newly planted field, one Cambridge Favourite plant had red steles and oospores in its roots.

In the experiment in which root tips of CF-2 were divided according to the possible site origin of the plants, the only positive bait test (one pot of six) was from samples of the yellow soil plants.

In the experiment to estimate the sensitivity of the bait test, symptoms were found in all samples to which diseased root tips had been added, regardless of

the quantity (Table 2).

No symptoms were found in bait plants from the random control pots or in any of the check samples prepared from the root tips of freshly dug plants.

DISCUSSION

The commercial stock of CF-1 must have been infected before delivery to the grower, indicating that the disease can be disseminated in certified planting stocks. The inability to detect symptoms despite detailed searching in the admittedly rotting stored planting stock further demonstrates the cryptic nature of the infection—either as a very low level in many plants or because it was present in only a few roots of some plants. Nevertheless, the bait test used was sufficiently sensitive to detect these low infection levels in the commercial stocks and to detect with near certainty the equivalent of one infected root system per 100 sampled.

Five weeks elapsed between starting the test and reading the results. In commerce, results would need to be available well in advance of the planting season. Where runner plants are stored cold after lifting, all or part of a stock could be sampled before the plants went into cold storage. If the plants were to be used shortly after lifting, a sample would have to be taken directly from the nursery beds before lifting. The 5-wk duration was based on earlier quantitative soil baiting tests (2), but more recent extensive supplementary tests showed that clear-cut symptoms of severe wilting, stunting, leaf discoloration (Fig. 1), severe root rotting, and typical red steles containing oospores were all often evident within 2 wk.

The success of the test, if developed for routine phytosanitary use, would primarily depend on the sample size and the number of infected plants. If diseased plants are randomly distributed in a large population, then the numbers of diseased plants in random samples would have a binomial distribution. The probability (P) of getting one or more diseased plants in such a sample is:

$$P = 1 - (1-d)^n,$$

where d is the proportion of infected plants in the population and n is the number of plants in the sample. Other secondary factors would be the severity of infection of each root or runner, the condition of the fungus in or on the plant (whether actively sporulating or present as oospores), and the resistance of the cultivar. The physical conditions of the test—temperature, light, and watering regimes—might be changed to further improve the speed of the test.

The resources and time to deal with samples are not great and a typical sample of 500 plants would require no more than four or five 12.5-cm-diameter

Table 1. Root-tip bait tests for red stele in three strawberry runner plant stocks

Stock	Runner plants		Pots (no.)		Bait plants	
	In each stock	Sampled	In each test	With infected bait plants	In each test	Infected
Cambridge Favourite (CF-1)	3,000	3,000	6	0	60	0
Cambridge Favourite (CF-2)	100,000	5,000	9	8	90	66
Cambridge Vigour (CV)	80,000	4,000	8	0	80	0
Control (compost alone)	2	0	20	0

Table 2. Root-tip bait tests for red stele disease in samples of root tips prepared by mixing root tips from infected and uninfected Cambridge Favourite strawberry runner plants

	Plants in each sample		Samples (no.)		Bait plants in 10 samples	
	Infected	Uninfected	Tested	With infected bait plants	Tested	Infected
Controls (Compost alone)	10	0	100	0
Checks	0	100	10	0	100	0
	1	99	10	10	100	99
	5	95	10	10	100	100
	10	90	10	10	100	100



Fig. 1. Bait plants of the Alpine strawberry cultivar Baron Solemacher after 5 wk in a root-tip bait test of three nursery stocks. Root tips in pots in center row were from plants from an infested nursery. Pots in left and right rows contain root tips from plants originating from two nursery sites that were not infested.

plastic pots and 20–25 bait plants. The work described here could clearly form the foundation for a routine test to monitor the health of strawberry planting stocks that would be far more sensitive, reliable, and reproducible than reliance on gross visual standards commonly in use.

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