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Strawberry: Developing a screening technique for *Colletotrichum acutatum* on strawberries

HORTICULTURE RESEARCH INTERNATIONAL

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REPORT FOR
HORTICULTURAL DEVELOPMENT COUNCIL

DEVELOPING A SCREENING TECHNIQUE FOR *COLLETOTRICHUM ACUTATUM* ON STRAWBERRIES

Contract No: SF 19b

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INTRODUCTION

Black Spot of strawberry, caused by the fungus *Colletotrichum acutatum*, has been a cause of concern to UK strawberry growers since it was first introduced to this country on runners of cv. Brighton, which were imported from California in 1983. That first outbreak was effectively controlled by eradicatory measures on all but one farm in Cornwall, but more recently further outbreaks have been reported following the importation of infected runner plants from mainland Europe. It is likely that these recent outbreaks can also be traced back to California via European nurseries who had imported mother plants of Californian cultivars, which are now widely grown in Southern Europe.

In the past black spot has normally been associated with the warmer strawberry growing regions of the world such as Florida, California, Australia, South Africa, New Zealand and, most recently, Italy and Spain. The optimum temperature for the fungus is 28°C and epidemics are usually associated with hot periods accompanied by high rainfall. It is splash dispersed so frequent rainfall or overhead irrigation will exacerbate the problem and experiments in the USA have showed that substantial dispersal occurred within 30 seconds during a rain shower and maximum incidence of rot in test plots occurred after showers of only sixteen minutes (Madden *et al*, 1992). Ripe fruit need remain wet for only a short time (c 6 hrs) near the optimum temperature for infection to occur (Ellis *et al*, in press)

Unfortunately for British growers the disease does not disappear at low temperatures and Ellis *et al* (in press) reported that it overwinters well on mummified fruit. Problems arise when it becomes established in the nurseries, particularly if these are raising everbearing cultivars, as it is impossible to remove all the fruits from these plants in the nursery bed and

the disease can rapidly spread from a few infected fruits to the leaves and stolons of other plants. Often infected plants will show no symptoms until the optimum conditions arise.

The current trend in the UK for increasing late season production, using either everbearers or 60-day plants, means that it is becoming more likely that growers will have fruiting plants at times when the environmental conditions are favourable to the disease. This will not cause problems so long as the Plant Health Propagation Scheme continues to supply our nursery industry with disease-free plants and strict monitoring of imported plants is observed. However, should these measures fail to prevent the disease from becoming established in this country then resistant cultivars will offer growers the simplest solution to the problem.

Winterbottom *et al* (1988 and 'in press') studied 50 Californian cultivars and breeding lines and found that over half were resistant. Furthermore, they concluded that the resistance was conferred by a major gene with resistance dominant to susceptibility. Since this material tested in California had not been bred with any deliberate intention of incorporating resistance to black spot it seems likely that the resistance is widespread and that there is a good chance of it being present among European cultivars. For the project reported here Winterbottom visited HRI East Malling to demonstrate and adapt the screening procedure while testing the susceptibility of European cultivars and HRI breeding lines.

MATERIALS AND METHODS

Cold stored plants of 15 cultivars available in the UK were obtained from a commercial nursery and 19 HRI breeding lines (Table 1) were propagated by pinning down runners in the glasshouse. Five isolates of *Colletotrichum acutatum* were obtained from Dr Roger Cook at the Central Science Laboratory, Harpenden. Four of these had been isolated from plants imported into the UK from Europe and one from strawberry fruits imported from Costa Rica.

To produce inoculum the pathogen was cultured on potato dextrose agar (PDA) and grown under continuous fluorescent light at 25°C ± 1°C. Conidia were harvested from PDA plates by flooding with 10 ml of sterile distilled water and agitating the culture with a glass rod. Aliquots (0.5 ml) of the conidial suspension were spread on PDA plates, harvested after 8 - 10 days as previously described, and then filtered through four layers of muslin to remove mycelial fragments. Conidial concentrations were adjusted to 1.5 x 10^6 conidia/ml for plant inoculation.

Prior to inoculation all plants were grown in 11cm pots in an unheated polythene house and given liquid feed on alternate days to encourage rapid vegetative growth. Just prior to inoculation all but the youngest 3 to 4 leaves were removed from plants which were 1.5 to 2.5 months old. All plant parts were spray inoculated to the point of runoff with a 1.5 x 10^6 conidia/ml suspension and were immediately placed in a dark growth chamber for 48 hr at 30°C ± 1°C, and 100% humidity was maintained by enclosing each plant in a polythene bag. Plants were removed from the growth chamber after 2 days and were subsequently maintained in a glasshouse at 30°C ± 5°C day and 22°C ± 5°C night temperature.

Photoperiod was not controlled but all experiments were completed in the period July to mid September.

Initially the pathogenicity of the four isolates derived from plants were tested by inoculating four Pandora plants (a known resistant - Faedi 1991) and four Elsanta plants (a known susceptible) with each. The Costa Rican isolate did not sporulate well and therefore was not used. For subsequent testing the most pathogenic isolate (IMI351247-1) was used throughout and two to four plants of the test cultivar or selection were inoculated on each occasion. Four Elsanta plants were included as susceptible standards at each inoculation date; two plants were inoculated and two were left uninoculated. Inoculations were repeated at least once using different plants and on occasions when the standard cultivar did not behave as expected, or where the results for the test plants were inconsistent, further test inoculations were done. Runners were rated daily for 15 days starting two days after inoculation. The disease severity rating (DSR) scale of Smith and Black (1987) was used on the runners and petioles: 0 = no lesions; 1 = lesion(s) <3 mm; 2 = lesion(s) 3-10 mm, not girdling runner; 3 = lesion(s) >10-20 mm, girdling runner; 4 = lesion(s) >20 mm, runner necrotic; 5 = youngest leaf wilted in absence of lesions, indicating crown infection; 6 = dead. This DSR scale was developed for use with the closely related fungus *Colletotrichum fragariae* and the two highest ratings on the scale (5 & 6) would not be expected when working with *C. acutatum)*. The mean DSR was calculated for runners for each genotype.

RESULTS

In the initial pathogenicity tests no symptoms were seen on Pandora plants but two isolates, from Cornwall and Kent, produced good symptoms on Elsanta. The Cornish isolate (IMI351247), originally from cv. Brighton, was the more pathogenic and was thus used in all subsequent tests.

For his original study in California, Winterbottom had rated genotypes with a mean DSR of 3.0 or greater 20 days after inoculation (DAI) as susceptible whereas those with a DSR less than 3.0 were classified as resistant. However, in the experiments reported here the results were much less consistent than had been experienced when using the same technique in either California or Italy (Winterbottom 1988, Faedi *et al* 1991). For example, the standard susceptible cultivar, Elsanta, had an overall mean of 2.77 with a standard deviation (SD) of 1.52 (Table 2), whereas in Italy the mean for Elsanta had been 3.99 and the SD 0.04 (Faedi *et al* 1991). Overall the standard deviations were variable and often large (Table 2) and consequently it was considered unsafe to classify the susceptibility of the genotypes on the basis of the means alone. Classification is thus based on a combination of the mean value and the distribution of the scores but in some cases the results were inconclusive. Classifications which are uncertain are indicated by a question mark (?) and any where the number of experimental plants was less than 10 should also be treated with some caution.

Despite the problems with symptom expression, it was possible to classify five lines as having a high level of resistance: Elvira, Honeoye, EM99, EM224, EM255; while six lines had a useful level of resistance but did show symptoms on some plants: Cambridge Favourite, Gorella, Pandora, Pantagruella, EM17 and EM290. Five lines were highly

susceptible: Elsanta, Redgauntlet, Tamella, EM237 and EM319; and the remaining 18 lines could not be classified with the same degree of certainty but 10 were probably resistant: Bogota, Cambridge Vigour, Hapil, EM20, EM90, EM320, EM407, LA937, LA969 and LA1043.

DISCUSSION

Overall the findings were much less clear cut and decisive than had been anticipated from results of earlier studies (Winterbottom 1988, Faedi *et al* 1991). Even Elsanta, the susceptible standard, which was found to be consistently highly susceptible in Italy (Faedi *et al* 1991), was recorded with a score of 4 on only slightly over 50% of the inoculated plants here and five plants (from 30) showed no symptoms at all. There are two possible explanations for this; firstly, it is possible that none of the isolates of *C. acutatum* available were particularly pathogenic compared to those used in California or Italy. Unfortunately it is not possible to test this hypothesis since plant health regulations prevent us from importing other isolates for comparison. A second explanation could be that the temperature and environmental conditions in the glasshouses where the testing was done were not consistently favourable to the disease. This second explanation seems the more likely one since this would also explain the inconsistency of the results during the course of the study. Although the glasshouses used here were no less sophisticated than those used in California or Italy the British weather is much less consistent and temperature fluctuations were unavoidable. This problem could be alleviated by using controlled environment cabinets but this would limit the scale of the experiments.

Breeding for resistance to *C. acutatum* is not currently an objective of the HRI programme but this study has shown that useful levels of resistance are present in both HRI breeding lines and Northern European cultivars. Should the disease become endemic in the UK then resistance screening could be incorporated into the breeding programme using the method described here but with closer control over environmental variation. Furthermore, two commercially acceptable resistant cultivars are already available to growers, namely Honeoye and Elvira. Pegasus may also have a useful level of resistance but requires further testing.

REFERENCES

Ellis, M.A., Madden, L.V. and Wilson, L.L. (in press). Studies on the epidemiology of anthracnose fruit rot of strawberry in Ohio. Acta Horticulturae.

Faedi, W., Bagnara, G.L., Turci, P., Winterbottom, C.Q. and De Clauser, R. (1991). Valutazione della suscettibilità ad antracnosi di diversi cloni di fragola. In: Convegno Nazionale sulla Fragola, Verona 8 November 1991. (Eds. Rosati, P., Fraccaroli, S. and Febi, A.) pp 261-269. Società Orticola Italiana.

Madden, L.V., Wilson, L.L., Yang, X. and Ellis, M.A. (1992). Splash dispersal of *Colletotrichum acutatum* and *Phytophthora cactorum* by short duration simulated rains. Plant Pathology, **41**, 427-436.

Smith, B. J. and Black, L. L. (1987). Resistance of strawberry plants to *Colletotrichum fragariae* affected by environmental conditions. Plant Disease, **71**, 834-837.

Winterbottom, C.Q. (1988) Resistance of strawberry to *Colletotrichum acutatum*. MS Thesis of Science in Horticulture. University of California, Davis.

Winterbottom, C.Q., Gubler, W.D. and Bringhurst, R.S. (in press). Comparative resistance of some California strawberry cultivars to one isolate of *Colletotrichum acutatum*. Plant Disease

Table 1. Pedigrees of HRI breeding lines

Line	Female parent	Pollen parent
EM17	Redgauntlet x (Wiltguard x Gorella)	Tioga
EM20	Redgauntlet x (Wiltguard x Gorella)	Tioga
EM22	Redgauntlet x (Wiltguard x Gorella)	Tioga
EM33	Redgauntlet x (Wiltguard x Gorella)	Tioga
EM90	Providence	Linn
EM99	Providence	Tioga
EM224	Honeoye	Hapil
EM237	Pegasus	North Star x (Redgauntlet x Merton Ruby)
EM255	Providence	Shuswap
EM290	Providence	Etna
EM317	[Redgauntlet x (Wiltguard x Gorella)] x Tioga	Elsanta
EM319	[Redgauntlet x (Wiltguard x Gorella)] x Tioga	Elsanta
EM320	Redgauntlet x (Wiltguard x Gorella)	SCRI seedling
EM404	Pandora x Bogota	Cambridge Late Pine x John Innes seedling
EM407	Pandora x Bogota	[Redgauntlet x (Wiltguard x Gorella)] x Tioga
LA934	Redgauntlet x (Wiltguard x Gorella)	Rainier
LA937	Redgauntlet x (Wiltguard x Gorella)	Totem
LA969	Tantallon	Shuksan
LA1043	Redgauntlet x (Wiltguard x Gorella)	Tioga

Table 2 Mean and maximum scores for *Colletotrichum acutatum* symptoms and susceptibility classification for 34 strawberry cultivars and breeding lines

Cultivar or Line	Number of plants	Mean score	SD	Max score	Plants with max score	Class
Bogota	8	0	0	0	8	RES
Cambridge Favourite	24	0.38	0.97	4	1	RES
Cambridge Vigour	8	0.25	0.71	2	1	RES
Elsanta	30	2.77	1.52	4	16	SUS
Elvira	16	0	0	0	16	RES
Gardena	2	1.5	2.12	3	1	SUS?
Gorella	14	0.5	1.16	4	1	RES
Hapil	6	0.17	0.41	1	1	RES
Honeoye	12	0	0	0	12	RES
Pandora	19	1.16	1.34	4	2	RES
Pantagruella	12	0.50	1.17	4	1	RES
Pegasus	25	1.20	1.38	4	3	RES?
Redgauntlet	36	2.56	1.68	4	17	SUS
Rhapsody	7	0	0	0	7	RES
Tamella	16	3.19	1.42	4	11	SUS
EM17	12	0.67	0.65	2	1	RES
EM20	6	0.67	1.03	2	1	RES
EM22	17	1.00	1.32	4	1	RES?
EM33	7	1.14	1.07	2	4	RES?
EM90	9	0.44	0.88	2	2	RES
EM99	14	0	0	0	14	RES
EM224	13	0	0	0	13	RES
EM237	15	3.93	0.26	4	14	SUS
EM255	12	0	0	0	12	RES
EM290	15	0.73	1.22	4	1	RES

Cultivar or Line	Number of plants	Mean score	SD	Max score	Plants with max score	Class
EM317	16	2.00	1.32	4	3	SUS
EM319	13	3.08	1.04	4	7	SUS
EM320	4	0.75	0.50	1	3	RES
EM404	10	1.3	1.49	4	1	SUS?
EM407	7	0.29	0.76	2	1	RES
LA934	5	0.80	1.10	2	2	RES?
LA937	6	0	0	0	6	RES
LA969	7	0	0	0	7	RES
LA1043	5	0	0	0	5	RES

SD = standard deviation