Strawberry: Host resistance to two-spotted spider mite

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RELEVANCE TO GROWERS AND PRACTICAL APPLICATION

Application

The aim of the project was to find sources of resistance to the two-spotted spider mite, *Tetranychus urticae*, which could then be incorporated into the strawberry breeding programme. Promising sources of resistance were found in a clone of the wild strawberry *Fragaria chiloensis* and in a few strawberry cultivars. However, crosses involving these sources have, so far, given disappointing results.

SUMMARY

Scope and objective of the project

The objective of the project was to search for sources of resistance to the two-spotted spider mite, an important pest of strawberry. The ultimate aim is to incorporate resistance to the mite into the strawberry breeding programme. The research concentrated originally on a clone of the wild strawberry, *Fragaria chiloensis*, an ancestral parent of the cultivated strawberry. This clone, coded WDL-40, from the west coast of North America, had shown promising results in preliminary tests with a collection of *F. chiloensis* clones, done at HRI East Malling prior to this project.

The project was extended to survey the large germplasm collection of strawberries, held at HRI East Malling, for resistance to spider mite. When promising sources of resistance were detected, crosses were made using these as parents, and the progeny were tested for resistance to mites.

Summary of results

Fecundity (number of eggs laid) of mites was used as the main measure of host plant resistance. Tests were also done on the development time of mites on the different plants, together with observations on mite mortality and the repellency of plants to mites.

The WDL-40 clone of *F. chiloensis* showed resistance to spider mite in many of the tests, though there was some variability in results between tests. Fecundity was often much lower than on the susceptible cultivar Elsanta, and in choice tests Elsanta was normally preferred by mites over WDL-40, so that substantially fewer eggs were laid on the latter. It was shown that the resistance was not due to increased hairiness of the leaf, or the thickness of the leaf cuticle.

When a selection of cultivars from the strawberry collection was tested, differences in susceptibility to spider mite were found. On several cultivars mites laid significantly fewer eggs than on Elsanta.

In an initial screen 46 cultivars were tested. They were then categorised on the number of eggs laid by mites over a standard period under standard conditions, in comparison to a known susceptible cultivar, Elsanta. Using this system, 9 were categorised as highly resistant, 11 as moderately resistant and 26 as susceptible. Further testing was done on the resistant cultivars and the more resistant of the moderately resistant class. However, these additional experiments revealed some variability in results between tests.

As the tests were done in a standard format, the major variable between tests on the same cultivar was the age of the plant. In order to test whether resistance to mites varied with plant age, one-year old plants that had overwintered were compared concurrently with juvenile runner plants propagated from them. The results suggested that fewer eggs were laid on the young runner plants than on the one-year old plants, though the effect was more apparent on some cultivars than others. The ranking of the cultivars with regard to level of mite fecundity was identical for both ages of plant.

Cultivars which showed evidence of the highest levels of resistance included Cambridge Prizewinner, Linn, Gorella and Oberschliessen. Crosses were made using some of these as parents, for example a cross was made between Cambridge Prizewinner and WDL-40. However, levels of resistance in the progeny of this and other crosses were disappointingly low, indicating that the inheritance of the resistance may be complex.

Action points for growers

No mite-resistant material is available from the strawberry breeding programme at present. The cultivars grown currently, such as Elsanta, are very susceptible to spider mites, and mite numbers can increase extremely rapidly under favourable climatic conditions. Growers therefore need to monitor their crops closely for spider mites and either take care to preserve native predators if they are present, by using a suitable pesticide programme, or release the exotic predator *Phytoseiulus*, if necessary after using a selective acaricide such as Apollo early in the season.

Practical and financial anticipated benefits

The project has indicated that there are potential sources of resistance to spider mite in wild clones and cultivars of strawberry. If these can be incorporated into the breeding programme then the resulting cultivars should be much less susceptible to mites than those in current use. This could make mite management much easier, as the slower reproductive rate of the spider mites would enable predatory mites to gain control much more quickly. Acaricide use would be greatly reduced, and any releases of predators could be done at a lower rate.

The search for a stronger, more stable, source of resistance to spider mite is continuing under MAFF-funded research. More clones of wild *Fragaria* species from various parts of the world are being evaluated, and also some species of *Potentilla*.

EXPERIMENTAL SECTION

Introduction

The two-spotted spider mite, *Tetranychus urticae*, is a very important pest of strawberries, as its feeding can severely debilitate plants. Chemical control is increasingly difficult because the mite has developed resistance to most of the acaricides approved for use on the crop, and few new acaricides are being marketed for use on soft fruit.

MAFF-funded research at HRI East Malling has shown that biological control using the exotic predatory mite *Phytoseiulus persimilis* can be effective, but it is expensive and the predators are often slow to gain control. On-going research at HRI is demonstrating the potential of native predators to provide biological control. However, the chances of these predators providing effective control would be greatly enhanced if the strawberry cultivars grown were less susceptible to the spider mite, as populations of the pest would develop more slowly.

If partial resistance to spider mite could be introduced into strawberry, acaricides should not be required and biological control by naturally-occurring predators may be sufficient, without the need for artificial releases.

Clearly, the first requirement is to find a source of strong resistance to the mite, and the purpose of this project was to screen strawberries for resistance. Much of the research concentrated on the extensive collection of strawberry varieties held at HRI East Malling, but testing was also done on a North American clone of the wild beach strawberry, *Fragaria chiloensis*, which is an ancestral parent of the cultivated strawberry. Where a promising source of resistance was found, tests were made on crosses which used that cultivar or clone as a parent. The research in this project is complementary to MAFF-funded research where a range of clones and species of *Fragaria* are being tested.

De Ponti (1977) showed that mite fecundity was a good measurement of resistance to two-spotted spider mite, so this was adopted as the standard test method. However, resistance can be manifested in other ways. For example plant material may be avoided (non-preference), so tests of mite 'run-off' were made and choice tests made where mites could choose between plant material. Another form of resistance occurs where development time of the mite is lengthened, and tests were also done to study this.

Materials and methods

Potted plants were used throughout, kept as far as possible in gauze-sided houses so that temperature and daylength were close to natural conditions. Where necessary in late summer, daylength was extended artificially by use of lights. Plants which were being compared were kept in identical conditions, and were all of the same age.

For tests of mite fecundity and development times, discs of 1.5 cm diameter were cut out of leaves, using a cork borer, and kept on moist filter paper connected by a wick to a reservoir of water. The discs were placed with the underside uppermost, and were replaced regularly during the course of experiments to avoid deterioration.

For oviposition tests, a teliochrysalis (the final resting phase before the mite becomes adult) and an adult male were placed on each leaf disc. When the female mite hatched from the teliochrysalis the male mated with her, and after a few days the female began to lay eggs. The number of eggs laid was then recorded every three days. There were ten replicate leaf discs for each plant tested. In addition to these 'no-choice' tests, some tests were made where female mites were placed on discs consisting of a half-disc of the test plant joined to a half-disc of Elsanta with a thin strip of sticky tape. The subsequent position of the females and the number of eggs on the two halves of the disc were recorded.

Studies of development times were made by placing two eggs on each leaf disc. Once one hatched the other was removed and development stage of the mite was recorded daily until it became adult.

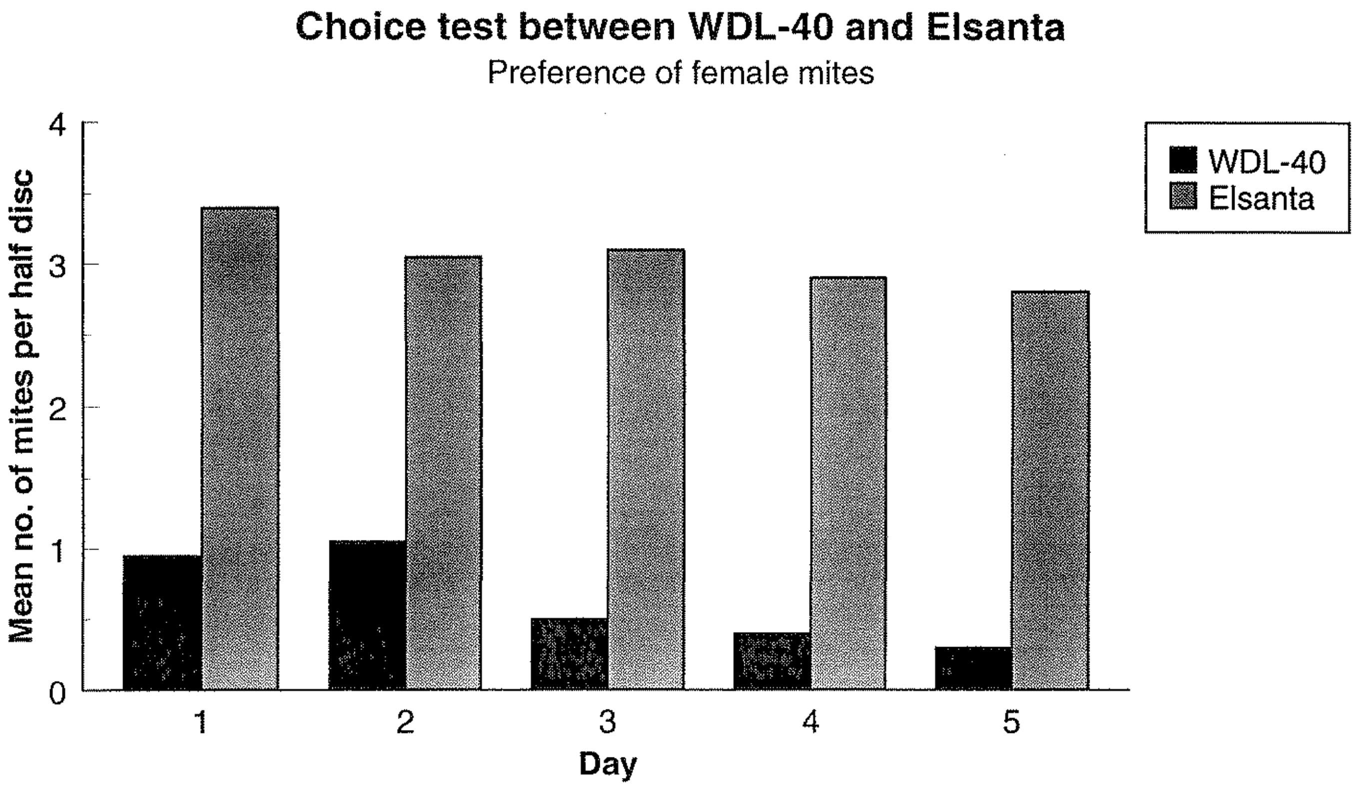
In all the fecundity and development tests, the cv. Elsanta was used as a susceptible comparison. The leaf discs were kept in a constant temperature room at 20 ± 2°C and a light: dark period of 16:8 hours. The mites used in the tests were taken from a laboratory culture kept under similar controlled environment conditions. The culture was established with mites taken from a variety of field sources, to maintain genetic diversity.

RESULTS

Clone WDL-40 and crosses involving this clone

In the first year of the project research concentrated on trying to confirm resistance to spider mite in the *Fragaria chiloensis* clone WDL-40, which had given promising results in preliminary tests (Easterbrook, unpublished). To investigate the inheritance pattern of any such resistance, the progeny from crosses made between WDL-40 and four clones of the cultivated strawberry were also tested for resistance to the mite. Further tests were then made on a second generation of progeny arising from crosses between plants of the F1 generation that showed lower mite fecundity, or from selfing of these plants (Table 1).

Figure 1


Choice test between WDL-40 and Elsanta
Preference of female mites
Mean no. of mites per half disc
0
1
2
3
4
1
2
3
4
5
Day
WDL-40
Elsanta

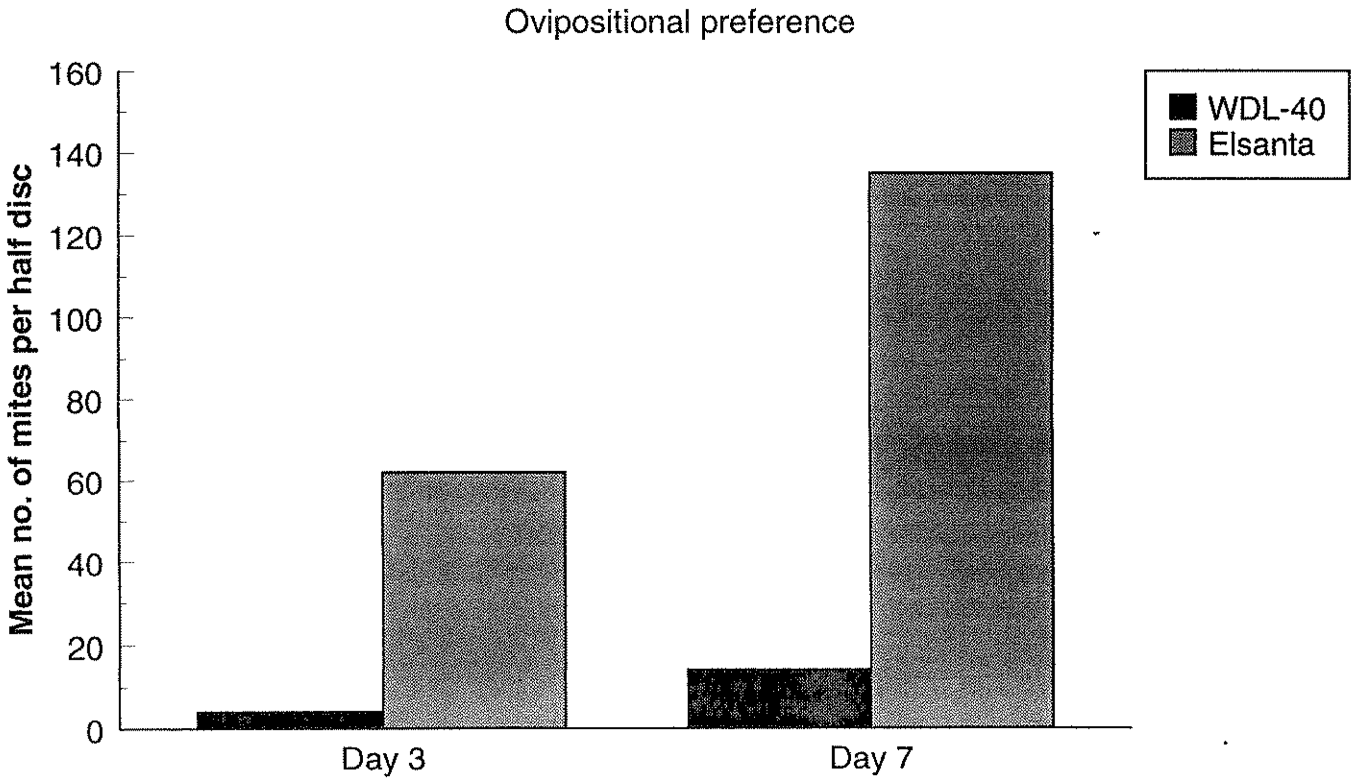

Ovipositional preference
Mean no. of mites per half disc
0
20
40
60
80
100
120
140
160
Day 3
Day 7
WDL-40
Elsanta

TABLE 1

Parents of F1 family ♂ ♀	F1 family named	F2 parentage	F2 family named
WDL-40 x Providence	MRX 24		
		Random crosses between 4 plants each of MRX 25, 26 & 27	MITE 1
WDL-40 x Selva	MRX 25	MRX 25 plant 8, selfed	MITE 2
WDL-40 x EM0013*	MRX 26	MRX 26 plant 1, selfed	MITE 3
WDL-40 x EM0038*	MRX 27	MRX 27 plant 6, selfed	MITE 4

* Breeding lines of the cultivated strawberry

On 63% of the 107 F1 plants tested, mites showed a statistically significantly lower level of fecundity than on Elsanta, with the greatest proportion in MRX 27 (71%). However, when the 280 F2 plants were tested very few plants showed reduced mite fecundity when compared to Elsanta, and when a second test was done on these plants, only one showed significantly lower fecundity for a second time. No correlation was found between the number of times mites rejected the leaf disc on which they were placed (a non-preference reaction) and fecundity on that disc.

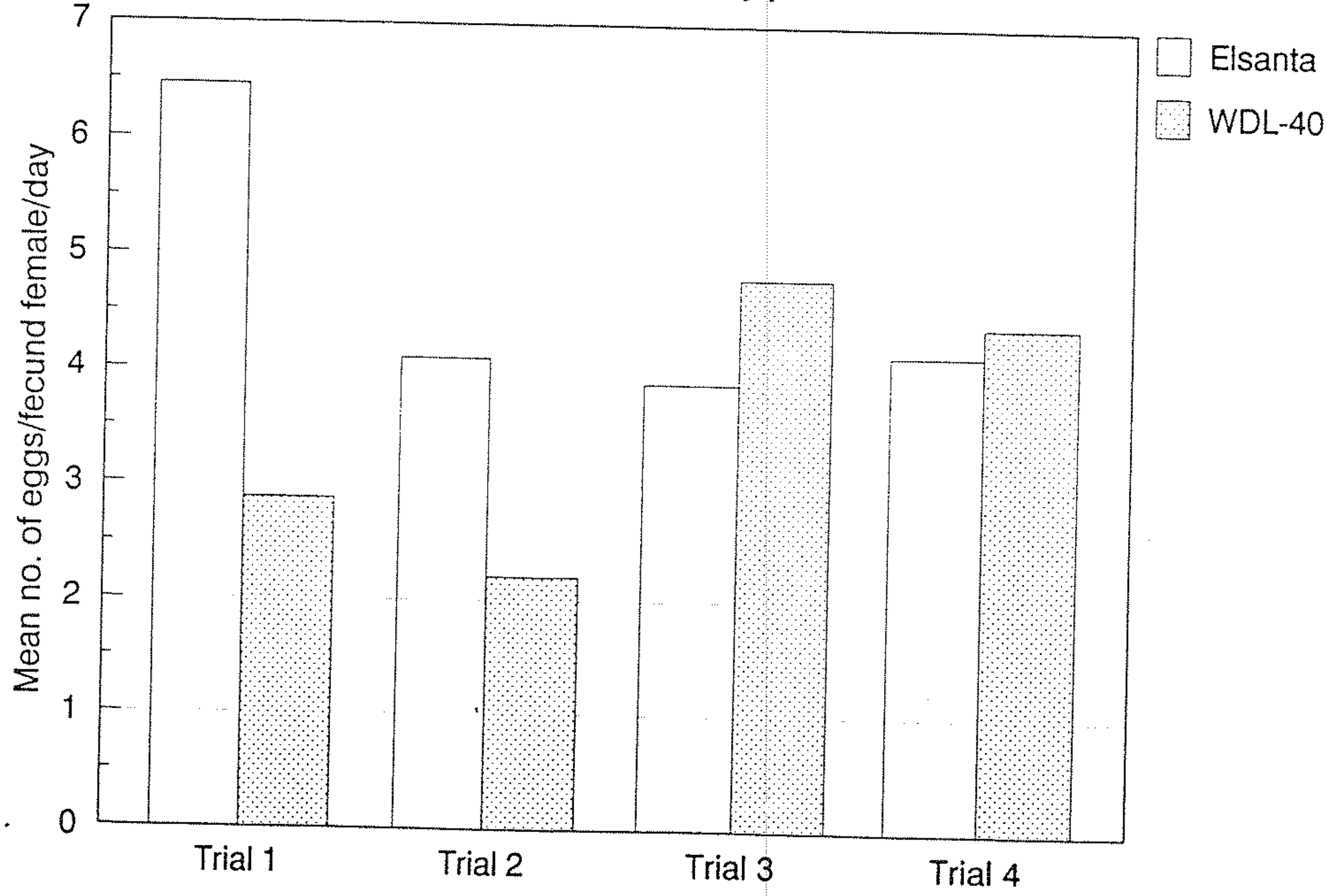
The first choice test using half discs of WDL-40 and Elsanta showed a highly significant level of non-preference ($P < 0.001$) by *T. urticae* for WDL-40 when compared to Elsanta (Fig. 1). However, when WDL-40 was paired with cv. Selva, another mite-susceptible cultivar, no significant difference was found. This might be explained, at least partially, by a preference for Elsanta over Selva in another choice test.

Choice tests were also done on the F2 plants used in the fecundity experiments. No significant differences from Elsanta were found in the number of mites found on each half disc, but there were significant differences in the numbers of eggs laid in some cases. However, these did not follow the same ranking patterns as in the fecundity experiments.

In the second year of the project further 'no-choice' fecundity tests were done on WDL-40, in comparison with Elsanta. Four trials were done, at intervals during the season, the first two using one year-old plants that had overwintered, and the second two using runners propagated in the current year. In the first two trials there was a significantly lower fecundity ($P < 0.05$) by mites on WDL-40 compared to Elsanta, but no difference was found in trials 3 and 4 (Fig. 2).

Figure 2. Fecundity of TSSM on Elsanta and WDL-40

Results from third 3-day period of trial



In a separate trial a test for non-acceptance of leaf material of WDL-40, in the form of mites leaving the leaf discs, did not show any increased 'run-off' compared to leaf discs of Elsanta. Results were the same whether adult female or deutonymph mites were used, and whether the mites had been reared on WDL-40 or Elsanta made no difference to the results. This contrasted with results from a preliminary test done prior to the start of the project, where there was much higher 'run-off' by mites from WDL-40 leaf discs (Easterbrook, unpublished).

Possible physical mechanisms for any resistance in WDL-40 were investigated. Leaves of WDL-40 are much hairier than cultivars such as Elsanta, with around 31 hairs per mm^2 compared to 4 per mm^2. Choice tests were made between half discs of normal WDL-40 leaves and those from which the hairs had been removed. There was a trend for more eggs to be laid on the hairy half, though this was only statistically significant on one day. Certainly, there was no evidence for the felt of hairs having a deterrent effect on mites.

Another possibility was that WDL-40 has a thick cuticle, which could hinder the penetration of the mouth stylets of the mite when feeding, and so reduce survival and oviposition. A comparison was made between small micropropagated plantlets of WDL-40 with a thin cuticle and slightly older plants when the cuticle had developed to normal thickness. There was no difference in survival of mites on the two types, and fecundity was higher on the plants with the well-developed cuticle, indicating that cuticle thickness is not a factor in acceptability of WDL-40 to mites.

Clone WBSP-14

Another clone of *F. chiloensis*, WBSP-14, was the most resistant of a series of clones tested by Shanks and Barritt (1984) in Washington State, USA. However, when we tested it mite fecundity was not significantly lower than on Elsanta, and was almost double that on WDL-40. This illustrates the need to test for resistance against different geographical populations of mites.

Testing the strawberry cultivar collection

In 1991 four strawberry cultivars from the collection were selected for tests on mite resistance, based on references in the literature of resistance, in field observations. In tests where female mites were given a choice between half discs of cv. Linn and the susceptible cv. Selva significantly fewer eggs were laid on Linn, whereas in another tests more eggs were laid on cv. Olympus than on Selva, while there was no difference in egg numbers in a Senga Sengana versus Selva choice.

Based on these results Linn was crossed with cv. Providence and the breeding line ES576 to give two families of seedlings, coded FX83/38 and FX 83/42, respectively. The seedling plants were tested in 1992, but although mite fecundity on some plants was significantly less than on the susceptible control cultivar Elsanta, the reductions in egg numbers were rather small.

In 1992, testing of strawberry cultivars from the large germplasm collection at HRI East Malling was expanded. Forty-six cultivars were screened for possible sources of resistance to spider mite, initially using the standard fecundity test. Those cultivars which appeared to show resistance in the initial test were given a repeated fecundity test and were also given a choice test with Elsanta.

The cultivars tested and compared with Elsanta in the initial fecundity test were categorised into 'resistant', 'moderately resistant', and 'non-resistant' classes by expressing mean fecundity on that cultivar as a percentage of that on Elsanta (Table 2). Of the 9 cultivars in the 'resistant' category, the lowest fecundity was recorded on Cambridge Prizewinner.

Repeat trials were then done for all cultivars in the resistant class, and those from the moderately resistant class showing the lowest fecundity levels. In these tests mite fecundity was higher than in the initial tests for all cultivars and although several cultivars still showed significantly lower levels of mite fecundity than Elsanta, the differences were much reduced from the initial tests (Table 3). Also, of the 9 cultivars in the initial resistant category, only Gorella and Madame Mutôt showed significantly reduced fecundity in the second test (Fairfax and Wiltguard were unavailable for re-testing).

One of the possible reasons for the discrepancies between the two sets of results was that the initial tests were done on leaf material from one-year-old plants that had overwintered, whereas the repeat trials were done on plants propagated in the current year. To test whether this was indeed the cause of the apparent variation in levels of resistance, further tests were carried out in the final year of the project.

TABLE 2

Results from preliminary trials of fecundity of *T. urticae* on a range of strawberry cultivars.

Resistant	Moderately Resistant	Non-Resistant	
$x < 50$	$50 < x < 75$	$x > 75$	
Cambridge Prizewinner+	Cacak Early	Aberdeen	Litessa
Cambridge Rearguard+	Cambridge Rival+	Allstar	Little Scarlet
Cheam+	Howard 17	Blakemore+	Marmion
Fairfax++	Huxley +	Cambridge Crimson	Merton Herald
Gorella+	Madame Lefebvre	Cambridge Late Pine	Merton Ruby
Harvester+	Merton Princess	Cambridge Profusion	Mrak
Madam Mutôt+	Merton Dawn+	Cambridge Sentry+	Suwanee
White Carter+	Montrose	Cardinal	Talisman
Wiltguard++	Oberschliessen+	Del Norte	Tamella
	Redgauntlet	Dukat	Tantallon
	Sparkle+	Glooscap	Triomphe de Tihage
		Kama	Tyee
		Linn	Vibrant

where $x = \frac{\text{cultivar mean}}{\text{control mean}} \times 100$

\+ re-tested
++ not available for re-test

TABLE 3

Re-trials of mite fecundity on strawberry cultivars from the germplasm collection

Cultivar	Mean no. of eggs/fecund female/day
Test A	
Blakemore	4.81
Elsanta	5.03
Test B	
Cambridge Prizewinner	5.19
Cheam	5.59
Harvester	5.80
Huxley	4.87*
Madame Mutôt	4.81*
Oberschliessen	3.70*
Elsanta	5.44
Test C	
Cambridge Rearguard	4.43
Cambridge Sentry	4.81
Merton Dawn	4.19*
White Carter	4.70
Elsanta	5.30
Test D	
	3.71*
Cambridge Rival	3.83*
Gorella	5.30
Sparkle	5.33
Elsanta	

* indicates a mean significantly less than Elsanta ($p<0.05$)

The first tests were in done in May, all on 1-yr-old plants that had overwintered; runners were not yet available. The results of fecundity tests on discs from leaves of these plants showed that oviposition on WDL-40, Linn, Gorella and Huxley was significantly lower than on Elsanta (Table 4). Numbers of eggs laid on other cultivars were not significantly different from Elsanta, except for Silver Jubilee and Cheam where fecundity was significantly higher.

TABLE 4

Fecundity of *T. urticae* on a range of strawberry cultivars (1-yr-old, overwintered plants)

Cultivar	Eggs/3 days
Elsanta	15.0
Linn	8.4
WDL-40	8.9
Gorella	9.7
Huxley	10.5
Cambridge Prizewinner	13.5
Harvester	13.6
Merton Dawn	14.3
Oberschliessen	14.5
Benton	14.7
Fairfax	14.9
Madame Mutôt	16.6
Cheam	18.9
Silver Jubilee	19.3
s.e.d. (110 df)	1.74

Further tests were done in August, when runner plants, which had been propagated from the overwintered 1-yr-old plants a few weeks previously, were available for comparison. The cv. Benton produced very few runners so was not tested. Overall, the difference in the numbers of eggs laid by mites on the plants of different age was not significant, but there was a consistent trend for fewer eggs to be laid on the runners than on the older plants (Table 5). Ranking of the cultivars for mite fecundity was identical for both ages of plant.

TABLE 5

Fecundity of *T. urticae* on a range of strawberry cultivars, comparing 1-yr-old overwintered plants with newly-propagated runners

	Mean egg numbers over 3-day period	
Cultivar	**1-yr-old plant**	**runner plant**
Elsanta	16.0	14.8
WDL-40	5.7	5.3
Fairfax	11.7	10.8
Linn	11.8	10.9
Oberschliessen	12.1	11.2
Gorella	12.3	11.4
Cambridge Prizewinner	12.3	11.4
Cheam	12.5	11.6
Silver Jubilee	13.2	12.2
Huxley	13.4	12.4
Harvester	14.7	13.6
Merton Dawn	15.0	13.9
Madame Mutôt	16.9	15.6

s.e.d. (184 df) 1.50

The greatest reduction in fecundity, compared to Elsanta, was seen on the *F. chiloensis* clone WDL-40. Cultivars with significantly lower oviposition than Elsanta were Linn, Fairfax, Oberschliessen, Gorella, Cambridge Prizewinner and Cheam: this was consistent for both the 1-yr-old and runner plants. Fecundity on other cultivars was not significantly different from Elsanta.

There were some differences in the ranking of cultivars with regard to mite fecundity, compared to the tests in May. Oviposition on the 1-yr-old plants of Linn and Gorella was higher than in the spring though still significantly lower than on Elsanta, and on Huxley egg numbers were also higher, with the difference from numbers on Elsanta greatly reduced in comparison to the results in May. In contrast, fecundity on Cheam and Oberschliessen was much lower than in the May tests.

Comparisons were also made of development time of the mite from egg hatch to adult on the two age classes of plant, over the same range of cultivars. Overall there was no significant difference for development times between 1-yr-old plants and runners, though for WDL-40 and Merton Dawn there was an indication that the mites might develop more slowly on the l-yr-old plants (Table 6).

TABLE 6

Development time of *T. urticae* from egg hatch to adult on a range of strawberry cultivars, comparing 1-yr-old overwintered plants with newly-propagated runners

	Development time (days)	
Cultivar	1-yr-old plant	runner plant
Elsanta	8.3	8.0
Fairfax	8.1	8.0
Huxley	8.0	8.0
Harvester	8.2	9.0
Madame Mutôt	8.3	8.1
Gorella	8.3	8.1
Cheam	8.3	8.2
Linn	8.6	8.4
Oberschliessen	8.8	9.4
Silver Jubilee	9.0	8.7
Cambridge Prizewinner	9.7	10.0
Merton Dawn	9.8	8.1
WDL-40	9.9	8.4
s.e.d. (159 df)	0.49	

Cambridge Prizewinner was the only cultivar that had a significantly higher mite development time than Elsanta for both ages of plant. WDL-40 and Merton Dawn had longer development times than Elsanta for 1-yr-old plants but not runners, while the reverse was the case for Harvester and Oberschliessen. Over the length of the project, there was evidence in some tests of a higher pre-adult mortality of mites on WDL-40 than on Elsanta and other cultivars.

CONCLUSIONS

Differences have been found between the fecundity, and to a lesser extent time of development, of two-spotted spider mite, *Tetranychus urticae*, on different strawberry cultivars and clones. In particular WDL-40, a clone of *Fragaria chiloensis*, has shown resistance to the mite in the form of reduced oviposition in both no-choice and choice tests, in comparison with a susceptible cultivar such as Elsanta. Some strawberry cultivars have also shown indications of resistance, including Cambridge Prizewinner, Gorella, Linn and Oberschliessen. However, in many cases results were not consistent between tests.

The study of biological parameters of the mite on plants of different age did not support the theory that this was the major cause of the inconsistent results. However, it is likely that changes in plant physiology/biochemistry are the reason. Studies on WDL-40 suggested that physical factors such as leaf hairiness were not a mechanism of resistance.

The results from testing the progeny of crosses suggest no major gene involvement, since highly resistant individuals were not found in either the F1 or F2 generation. Quantitative inheritance seems the more likely explanation but more resistance would still have been expected in the F2 generation. However, it is possible that the susceptibility testing procedure used was not sensitive enough to detect small genetic differences, particularly among the more susceptible seedlings. It could be that the additive effect of resistance genes is only detected once a certain threshold level is reached and that few individual seedlings reached that threshold. Furthermore, all seedlings were tested as young plants when they may have been more susceptible anyway. A programme of backcrossing to WDL-40 may reveal more about the genetics of resistance, leading to a breeding strategy. In the meantime the project has indicated some cultivars that could be used in the breeding programme to reduce the susceptibility to spider mite that is prevalent in the cultivars currently in use. The search for a strong source of resistance will continue in MAFF-funded research, studying other clones of wild *Fragaria* species, and also some *Potentilla* species.

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