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Breeding for Resistance in California Strawberry to Verticillium dahliae

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This article will focus on breeding resistant strawberries to the fungus Verticillium dahliae, common name Verticillium wilt. This is a serious fungal disease that can result in a loss of 50% or more of a strawberry harvest when grown in infested soil .[6] *The main goal is to provide an example of how a cultivar can be made more resistant to a plant pathogen with the use of plant breeding methods.*

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Overview

Since 1930 the University of California, Davis, has been developing strawberry cultivars that are adapted to the agricultural industry and regional farms. Developing cultivars that require fewer inputs are of significant economic importance in agronomy. Developing a crop resistant to a disease is beneficial for horticulturists since less labor and chemicals are needed for a high yield.[6]

In commercial strawberry cultivars, complete resistance to *V. dahlia* is extremely rare. The majority of Californian strawberry cultivars are highly susceptible to it.[2] Over the last 18 years of plant breeding for strawberry cultivars with a high degree of resistance and horticultural traits, strawberry cultivars with at least moderate resistance grew to 53.8% of the strawberry germplasm being measured in the study. Each plant was rated from 1 to 5 based on symptoms of *V. dahliae* in comparison to control plants over the length of the study.

- The lowest ranking was 1 meaning severe stunting or death
- A ranking of 3 indicated moderate damage and signs of tolerance
- The highest ranking was 5 indicating no disease symptoms

Introduction

The current process of increasing yield by the use of soil fumigation was first demonstrated in 1953 with the use of chloropicrin.[1] However, broad spectrum soil fumigants are costly to apply and reduce the profitability of the harvest.[9] The use of preventative fumigants may no longer be an option for the control of soil borne disease due to the possibility of legal restrictions. [4] Soil fumigants require treated soil to be covered with gas-proof sheeting (polyethylene or vinyl) for at least 24 to 48 hours after treatment. Planting cannot take place for an additional 2 to 3 weeks. [9] Even when fields are sprayed with a fumigant mixture of methyl bromide and chloropicrin (MBC) *V. dahliae* reemerges in the following year. [7] Due to the above factors, research experiments have been carried out at the University of California Davis (UC Davis) with the ultimate goal to develop highly productive commercial lines of resistant strawberry varieties that no longer depend on fumigant application of the soil. [4]

An example of how *V. dahilae* disrupts productivity can be seen when comparing the healthy strawberry plant in Figure 1 with Figure 2 that shows a field infested with Verticillium wilt disease.

Fig. 1 Healthy strawberries
(Image used with permission from Douglas V. Shaw, UC Davis)

Microsclerotia are the reproductive spores of *V. dahilae* that can live in the soil for 25 years making crop rotation useless. *V. dahliae* can spread quickly to new areas by seed, tools, machinery, and by infected roots.[9] The fungus causes the outer leaves of strawberries to become necrotic and the growth of new leaves are greatly reduced. [1] A variety of weeds can act as carriers of *V. dahliae* making eradication difficult.

Fig. 2 A strawberry field with a high concentration of *V. dahliae* at 30 ms/gram. The field above shows various levels of resistance from little injury to death.
(Image used with permission from Mark Bolda,, UCCE).

Fig. 3 Strawberry plants showing various levels of resistance to Verticillium wilt. The two plants on the left are susceptible to the disease as indicated by the black roots. The plant in the center shows moderate resistance to the disease. The two plants to the far right are strawberry plants that show a high level of resistance (UIUC, Image used with permission from J.L. Maas).

Mechanism of Resistance

Resistance to *V. dahliae* shown by certain strawberry lines is a polygenic trait controlled by both additive and dominant genes which can be observed in the bell curve pattern of Figure 4. [6] Strawberry plants have gained resistance to *V. dahliae* by restricting the flow of the infectious structures through vegetative matter. Within susceptible plants, *V. dahliae* is easily transmitted within the stolons of strawberry plants, resulting in high levels of damage despite the low prevalence of the disease. The possession of this trait reduced yield loss in susceptible plants from 73 - 75% down to 7% -15% for resistant plants. The ability to tolerate the infection also prevents the fungus from spreading from mother plants to runners. As indicated by petiole assay 80.7% infection rate was present in susceptible mothers and 59.8% infected runners. In comparison to resistant mothers who had 77.3% infection rate, but passed the disease to only 25.1% of the infected runners. [5]

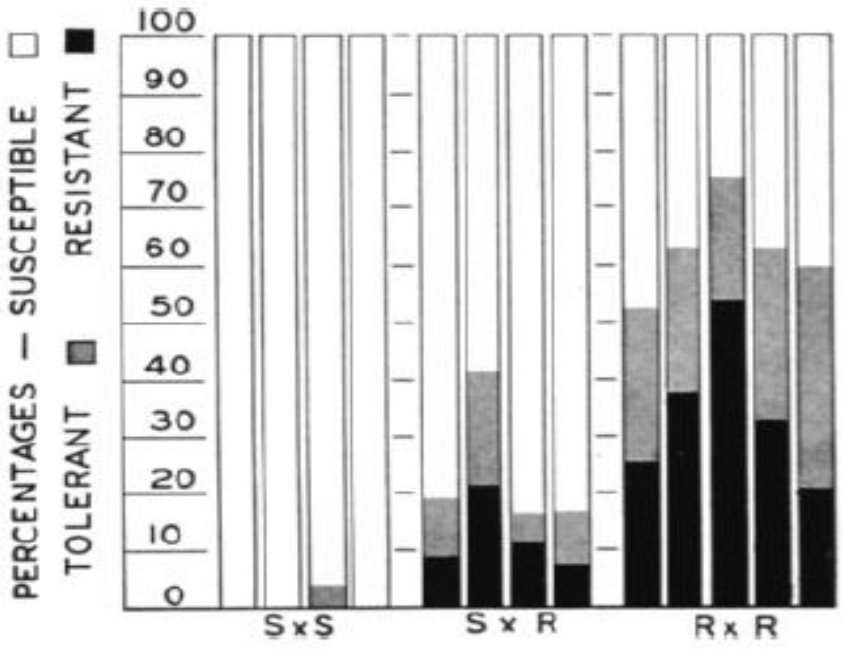

Fig. 4 The percentage resistant and tolerant strawberries from susceptible (S) and resistant (R) plants. More than 2,300 seedlings were tested in this study (Image credit Stephen Wilhelm, UC Berkeley).

Breeding for Resistance

Now that we have described the impact of *V. dahliae* on strawberries and the importance of researchers developing lines that are resistant for farmers to use, here are the details of how resistance was increased. Plot-mean evaluations of commercial varieties have shown that the genetic basis for the *V. dahliae* resistance trait in strawberry cultivars is highly heritable.[2] This means that it is possible to breed gene traits responsible for resistance and the increase can be shown in Figure 4 by statistical analysis. To determine the value of any plant in the study, a regression line graph was used that charted traits of interest such as disease progression and yield. This was done to measure the level of resistance in the test plot being studied and determine which plants should be crossed together to produce offspring with increased resistance levels illustrated in Figure 5.[6]

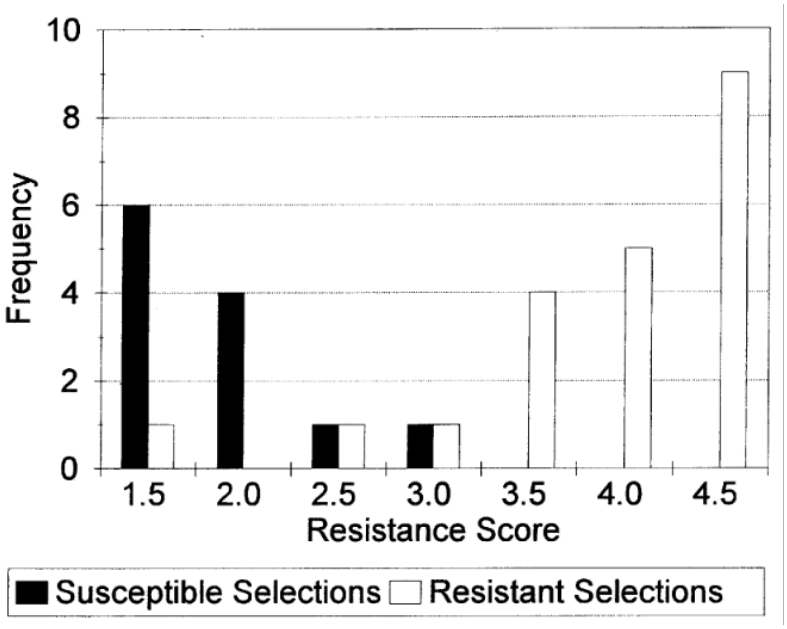

Fig. 5 The bidirectional selection for resistance as well as susceptible strawberry plants. (Image used with permission from Douglas V. Shaw, UC Davis)

Each trial year, a percentage of strawberry lines that had been graded with at least a 3 for moderate resistance were determined. Strawberry plants that had been graded at least a 3 or greater rating were included in the next year's breeding program. [6] The majority of these resistant plants had dark green glossy leaves; a visual characteristic that was found to be correlated with resistance. [8] Breeding for resistance of *V.dahliae* has had the largest significant result with strawberry lines that showed a moderate resistance of 3 or greater. [6]

In contrast, strawberries with a score of 2 or less were eliminated unless they contained excellent horticultural characteristics. A program of continuous improvement was enacted where progeny who had a parent with a rating score of 2 or less had fewer seeds or runners saved, The remaining germplasm had a moderate to high level of resistance which was used to enhance the overall breeding program. [6]

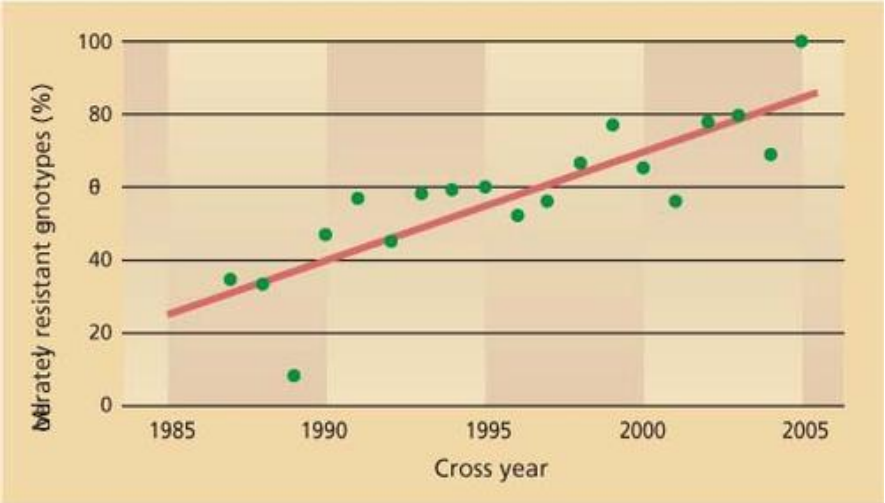

Fig. 6 Shows results from breeding experiments from 1987 (original germplasm)) to 2005. This regression line demonstrates the change in percentage of genotypes with at least moderate resistance
(Image used with permission from Douglas V. Shaw, UC Davis, 2010).

Evaluating resistance in test plots

A strawberry plant's resistance rating and yield were plotted each year on a regression line graph as seen in figure 6. A research field was designated to each of the tests performed. There are 2 methods of evaluating resistance that were used in this study: Runner inoculation and direct genotype evaluation.

Runner inoculation

During this study the researchers were looking for single parents to find which of them has moderate to high levels of resistance to verticillium. Multiple runners were taken from strawberry plants in order to form a family group for testing the level of genetic resistance to the disease. The submersion of runners within a medium containing the fungal spores is the primary method for family selection within the study (see Figure 7). So what Shaw's team did was took a sample of runners from each family and inoculated them with the verticillium fungus. After treatment the strawberry runners were then transplanted into an assigned test plot. This test allows for the evaluation of traits with minimal genetic influence.

This direct root inoculation technique allows for greater amounts of plant material to be screened because several clone plants can be obtained in the form of runners. In this way, the researchers were able to replicate the strawberry genetics being tested for resistance, since each runner clone would have the exact same genetic makeup. These clone plants can then be evaluated for resistance by progeny tests. This technique has the most thorough inoculation procedure and therefore allows for a high probability of disease transmission. The issue with this method is the low amounts of genetic diversity within the strawberry cultivars being tested. This narrow view is unfavorable due to the high level of diversity present that leads to resistance.[3]

Direct genotype evaluation
A second test performed was the direct screening of genotypes by inoculating peat pots containing the experimental seedlings. The seedlings were derived either from the original pure strains to use as a control or from previous breeding efforts. Peat pots were placed in a 1 cm deep pan holding 1 liter of inoculant solution until they had absorbed about 15 ml. The peat pots were randomly distributed for planting in an assigned test plot.

This is beneficial because individual specimens can be measured separately. This allows for the quick detection of resistant types. However, the plants that show no signs of disease may have escaped treatment due to the heterozygous distribution of spores in the inoculant.[3]

Fig. 7 A petri dish showing a colony of *V. dahliae.* The black specks are Microsclerotia; the reproductive particle of the fungus.
(Image used with permission from Steven Koike, UCCE).

All test plots from either evaluation method were graded on the 1 to 5 scale for disease symptoms; 4 to 7 times during the trial year at approximately 3 week intervals. The final score for each individual seedling across the year was calculated by taking the average of all ratings during the time of the research period .[3]

All plants involved within the study were tested for *V. dahlia* by petiole assay in order to validate the presence of infection. Petiole assay is the culturing of a cross section of a plant's vascular system on agar media plates to determine the rate of infection within the test plots. All plates showed a positive result of *V. dahliae* during this study.[2] The process used by UC Davis in this e-library article proves that a greater level of resistance to fungal disease can be obtained by conventional plant breeding practices. Development of this technique of verticillium resistance can be used in other horticultural crops such as tomatoes, lettuce, potatoes and Chrysanthemum.[1] An additional benefit found by Wilhelm is *V. dahliae* resistant plants also exhibit a cross-resistance to another dangerous plant disease, powdery mildew.[8]

Summary

In this article it has been seen that crop loss by *V. dahliae* can be reduced by breeding resistant cultivars of strawberries. This has the additional benefit of reducing soil fumigation which is costly to apply and requires special equipment. During the study resistance steadily increased to 53.8% of the cultivated germplasm having at least moderate resistance. This was achieved by rating plants by disease progression by runner inoculation and direct genotype selection. Those with the highest average resistance were bred together based on linear regression of the data. Although complete resistance to *V. dahliae* has not been developed, strawberries resistant to this pathogen may become an important tool in growing strawberries and other horticultural crops in areas infested with high levels of *V. dahliae*.

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