Gene-for-gene relationships between strawberry and the causal agent of red stele root rot, *Phytophthora fragariae* var. *fragariae*.

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Gen-om-gen relaties tussen aardbei en de veroorzaker van roodwortelrot, *Phytophthora fragariae* var. *fragariae*.

promotor:prof. dr. ir. J.E. Parlevliet
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Gene-for-gene relationships between strawberry and the causal agent of red stele root rot, *Phytophthora fragariae* var. *fragariae*

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On the cover:
A new CPRO-DLO strawberry cultivar with resistance to crown rot (Phytophthora cactorum), and Verticillium and with multiple resistances to red stele (Phytophthora fragariae). This cultivar is currently under release.

Bij de omslag:
Een nieuw CPRO-DLO aardbeiras met resistentie tegen stengelbasisrot (Phytophthora cactorum) en verwelkingsziekte (Verticillium) en met meerdere genen voor resistentie tegen roodwortelrot (Phytophthora fragariae). Dit ras wordt momenteel geïntroduceerd.

STELLINGEN

1. Het is nu mogelijk om aardbeirassen te veredelen met gespecificeerde combinaties van genen voor resistentie tegen de veroorzaker van roodwortelrot, *Phytophthora fragariae* var. *fragariae*.

   dit proefschrift

2. De kracht van het gebruik van moleculiare merkers voor de veredeling op resistentie tegen *Phytophthora fragariae* var. *fragariae* kan pas echt benut worden wanneer zulke merkers voor alle gewenste resistentiegenen beschikbaar zijn.

   Haymes KM, Van de Weg WE, Meulenbroek EJ, Vosman B, Maas JL, Den Nijs APM (1997c) Conservation of linkage of RAPD and SCAR markers to the *Rpf1* resistance gene for *Phytophthora fragariae* in strawberry. In Haymes KM (ed) Molecular genetic studies in *Fragaria* species: *Agrobacterium*-mediated transformation and fine mapping of the *Phytophthora fragariae* resistance gene *Rpf1*. CPRO-DLO, Wageningen, pp. 65-82

3. Het belang van het gemiddelde aantal oosporen van *P. fragariae* in de onderste 8 mm. van een wortel als parameter voor resistentie kan pas onderzocht worden indien deze parameter niet verstrengeld is met het percentage geïnfecteerd wortelweefsel.

   Milholland RD, Cline WO, Daykin ME (1989) Criteria for identifying pathogenic races of *Phytophthora fragariae* on selected strawberry genotypes. Phytopathology 79: 535-538

4. De conclusie van Senanayake & Bringhurst (1967) dat de door hen onderzochte octoploïde *Fragaria* soorten *F. chiloensis* en *F. virginiana* eenzelfde genoomstructuur hebben, vindt geen ondersteuning in hun data.

   Senanayake YDA, Bringhurst RS (1967) Origin of *Fragaria* polyploids. I. Cytological analysis. Am J Bot 54: 221-228

5. Het gebruik van het werkwoord 'zijn' bij het beschrijven van de resistentie van planten voor (a)biotische factoren is ongewenst.

6. In onderzoek naar de overerving van resistentie tegen *Venturia inaequalis* (schurft) in appel dient een flexibele aantastingsdrempel gebruikt te worden waarbij de getalswaarde van de drempel afhangt van het niveau van de onderzochte resistentie.

   Shay JR & Hough LF (1952) Evaluation of apple scab resistance in selections of *Malus*. Am J Bot 39: 288-297

   Hough, LF, Shay JR, Dayton DF (1953) Apple scab resistance from *Malus floribunda* Sieb.. Proc Am Soc Hort Sci 62: 341-347

7. Het ontbreken van een gen-om-gen model bij het appel-*Venturia inaequalis* pathosysteem belemmert een efficiënte exploitatie van fysiospecifieke resistenties door veredelaars.

8. Fysiospecifieke resistentiegenen kunnen soms ten onrechte beschouwd worden als modificerende genen van andere fysiospecifieke resistentiegenen.

   Rousselle GL, Williams EB, Hough LF (1974) Modification of the level of resistance to scab from the *Vf* gene. Proceedings of the XIXth Intern Hort Cong, Warszawa

9. "Doorbroken" fysiospecifieke hoofdgenen kunnen voor 'quantitative minor genes' aangezien worden indien in genetisch onderzoek een mengsel van fysio's gebruikt wordt.

   Parlevliet JE (1983) Can horizontal resistance be recognized in the presence of vertical resistance i nplants exposed to a mixture of pathogen races? Phytopathology 73: 379

10. Mengselmodellen ("mixed models") worden nog te weinig toegepast in genetisch onderzoek.

    Jansen RC (1995) Genetic mapping of quantitative trait loci in plants - a novel statistical approach. CPRO-DLO, Wageningen, 109 pp.

11. Het slikken voor zoete koek kan gevaarlijk zijn.

Stellingen behorend bij het proefschrift "Gene-for-gene relationships between strawberry and the causal agent of red stele root rot, *Phytophthora fragariae* var. *fragariae*" door W.E. van de Weg, 23 juni 1997

ABSTRACT

Red stele (red core) root rot is the major soil-borne disease of strawberries (*Fragaria spp.*) in many areas with cool, moist soil conditions. It is caused by the soil-borne fungus *Phytophthora fragariae* var. *fragariae*. Red stele is a quarantine disease in Europe with a zero tolerance for commercial stock plants. Any lot of such plants with even just a trace of the disease has to be destroyed. The plot on which the plants were harvested has to be abandoned for commercial strawberry propagation forever. Consequently, this disease is not only a menace to growers, but also to nurseries. Red stele has been successfully combatted by resistant cultivars in the USA and Canada. In Western Europe, no cultivars with an effective resistance are available. The Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in The Netherlands initiated the breeding for resistance in 1968 and a number of advanced resistant selections has been produced. Their horticultural value remained behind that of the successful CPRO-DLO cultivar Elsanta, so none of them was as yet released as cultivar. The breeding for resistance has thus far been complicated by a general lack of insight in the genetics of resistance as well as the absence of a reliable resistance test. The research described in this thesis aimed to increase opportunities to create elite cultivars with red stele resistance by overcoming these complications.

Tests for resistance. Resistance tests were developed for use under controlled environmental conditions. Their main innovation is their systematic account for incomplete as well as for complete resistance. This is achieved by comparing the level of disease of the tested genotype with that of a universally susceptible reference cultivar. The tested genotype is supposed to possess resistance when it is significantly less diseased than the reference cultivar. This approach thus includes the introduction of a *flexible* disease threshold to distinguish resistance from susceptibility.
An other innovation deals with the assessment of segregation ratios for resistance in inheritance studies. The percentage of genetically resistant descendants is estimated by adjusting the proportion of relatively healthy seedlings for susceptible descendants which escaped from (severe) infection.
These two innovations led to the identification of some individual resistance genes, including one for moderate resistance of the cultivar Cambridge Favourite.

Genetics of resistance. A genetic model was developed. It explains the resistance of strawberry cultivars to isolates of *P. fragariae* by the interaction of five resistance and five avirulence factors. This so called gene-for-gene (GFG) model is similar to the one developed by Flor for the flax-*Melampsora lini* relationship. Inheritance studies on two of the five resistance factors revealed that each of them was based on a single gene, which

were designated *Rpf1* and *Rpf2*. This study thus showed that race specific resistance in strawberry for *P. fragariae* is single-gene based. The model is the first GFG-model for a soil-borne fungus, while its genes are the first identified resistance genes in strawberry.

The establishment of a gene-for-gene relationship is a major step in the elucidation of the genetics of resistance to *P. fragariae*. The proposed GFG-model can improve the efficiency of breeding programs since it allows the combining of consciously chosen resistance genes into cultivars. The model makes it also possible to develop a universally applicable differential series of strawberry genotypes, which series is essential for the identification of fungal races.

The tests for resistance and the genetic model as proposed in this thesis can be a positive contribution in the breeding of red stele resistant cultivars, due to which they are fully incorporated into the strawberry breeding program at CPRO-DLO. The results of this research can thereby ultimately lead to a more reliable, paying, and environmentally acceptable culture of strawberry in red stele infested areas.

CONTENTS

OUTLINE OF THE THESIS

This thesis is the result of research intended to support the breeding of strawberry cultivars resistant to the red stele disease at CPRO-DLO, The Netherlands. It had in view to contribute to the elucidation of the genetics of resistance, to provide the breeders with a resistance test, and to assemble a series of fungal isolates by the use of which breeders can discern among the various desired resistances. In addition, the research aimed at the initiation of an international set of differential strawberry genotypes. Problems anticipated in this study were related with the polyploidy of strawberry, the accuracy of disease assessment, and the occurrence of incomplete and moderate resistance.

In Chapter 1 an introduction is presented on the strawberry, its pathogen *Phytophthora fragariae*, and its disease red stele root rot.

Chapter 2 presents the first discovery of a monogenic resistance to *P. fragariae* in the cultivated strawberry, which was recognized by analyses of field trials.

Chapter 3 describes the development of a test for resistance which is performed under controlled growing conditions. The main issue addressed is the interpretation of disease severity data which leads to an adequate discernment between resistant and susceptible genotypes.

Chapter 4 applies this test for resistance to the USA and Canadian differential series of strawberry genotypes, which were tested against a series of 13 North American fungal isolates.

Chapter 5 proposes a gene-for-gene model for five resistance factors in the strawberry and five avirulence factors in the fungus based on the data of chapter 4. It also applies this model to data from the literature.

Chapters 6 and 7 describe inheritance studies which demonstrate the monogenic basis of the resistance factors 1 and 2, which is a prerequisite for the validity of the above described gene-for-gene model.

Finally, The General Discussion aims to integrate the results of this thesis with some recent research developments, and indicates opportunities for research on molecular markers, the results of which should further facilitate the breeding for resistance.

I. AN INTRODUCTION TO THE STRAWBERRY, THE RED STELE DISEASE, *PHYTOPHTHORA FRAGARIAE*, AND RESISTANCE

THE STRAWBERRY

Culture. Strawberry is one of the most important soft fruit crops. In western Europe, about 800.000 metric tons are produced annually, of which Spain and Italy account for half of the production. In The Netherlands, strawberry yields about 45.000 metric tons on 1500 hectares with a market value over 150 million guilders. Besides, stock plants are produced on around 400 hectares and are marketed world wide. One of the leading cultivar in western Europe is Elsanta, which covers 90% of the Dutch acreage, and which was bred at CPRO-DLO (IVT). All cultivars grown in western Europe, including those of CPRO-DLO, are susceptible to red stele root rot.

Species, ploidy levels, and genome structures. The strawberry exists as di-, tetra-, hexa-, and octoploid species which frequently and spontaneously hybridize across ploidy levels. This study focuses on the cultivated strawberry *Fragaria x ananassa* and its founding species *Fragaria chiloensis* and *Fragaria virginiana*. The genome structure of these three octoploid (2n=8x=56) species is still unresolved. Structures that have been suggested on the basis of cytological studies are: AABBBBCC and AAA'A'BBBB. Recently, a molecular linkage map was initiated in the diploid strawberry. When applied to the octoploid strawberry, such research may ultimately resolve the genome structures of these species. The currently presumed genome structures allow for di- and tetrasomic gene segregation within a single progeny.

(Fedorova 1946; Senanayake & Bringhurst 1967; Staudt 1989; Bringhurst 1990; Galletta & Maas 1990; Haymes 1993; Davis & Yu 1997)

RED STELE

Relevance & occurrence. Red stele (red core) root rot is the major soil-borne disease of strawberries in many areas with cool, moist soil conditions. It is caused by the soil-borne fungus *Phytophthora fragariae* and was first observed in the Larnarkshire district in Scotland in 1920, where it changed a flourishing export industry of 1500 acres into 'a skeleton of its former self' (Alcock & Howells 1935). Since it has been recorded in

Australia, Canada, Japan, Lebanon, New Zealand, the Soviet Union, and the United States of America. In Europe the disease occurs in Austria, Bulgaria, Denmark, France, Germany, Ireland, Italy, Sweden, Switzerland, The Netherlands, and the United Kingdom.

Quarantine organism. In Europe, red stele is a quarantine disease with a zero tolerance. Any lot of stock plants with even just a trace of the disease has to be destroyed. The plot on which the plants were harvested has to be abandoned for strawberry propagation for ever. Consequently, red stele is not only a menace for growers, but also for nurseries.

Spread. Red stele is frequently introduced into new areas by infected plants, whose infections were not detected during propagation. Subsequent spread within an area is favoured by moving water, e.g. slopes and tracks. In susceptible cultivars, the disease may spread rapidly and soon render the entire planting worthless.

Combat. In the USA and Canada, resistant cultivars have shown to combat the disease effectively. Early attempts in Europe (Scotland) to create resistant cultivars with superior horticultural value were not successful. A reason for this might have been differences in view among breeders and phytopathologists on the value of incomplete resistance.

Other practices to combat the disease are the use of clean planting stock and fungicides, and cultural practices such as improvement of drainage and culture on raised beds. Sanitary certification schemes have been helpful in the production of healthy commercial stock plants in The Netherlands as well as the UK and the USA, although this measure could not fully prevent the further dissemination of the fungus.
Fungicides which are widely used are Ridomil, Ridomil-Gold, and Alliette. Since this fungus can build up resistance to them, their effectiveness usually declines in time. Besides, their is increasing opposition from the public to their use. Methylbromide and chloropicrin are still widely used in California, but will soon be completely forbidden.

PHYTOPHTHORA FRAGARIAE VAR. *FRAGARIAE*

Host range. *P. fragariae* was identified by Hickman (1940) on strawberry, and has recently been subdivided into the varieties *Fragariae* and *Rubi*. The former is pathogenic to all species of strawberry and was found to be slightly infectious to some other members of the *Rosacea* including *Dyras*, *Geum*, *Potentilla*, and *Rubus*. *P. fragariae* var. *rubi* is believed to be pathogenic to raspberry only.

Biology. The fungus infects healthy roots by zoospores. These swim to young laterals and rootlets as well as to the region of the root tips of main roots. There, they encyst and produce germ tubes, which penetrate the epidermis. Next, hyphae grow towards the pericycle and phloem, from where they grow along the stele upwards to the crown. In the meanwhile, zoosporangia are formed vegetatively at the root surface, and oospores are formed sexually alongside the stele. Roots begin to rot a few days after infection and eventually, rotted roots containing oospores become incorporated into the soil, where they can remain viable for 15 years. Shortly after their germination, oospores produce zoosporangia which ultimately release infectious zoospores.

(Hickman 1940; Bain & Demaree 1945; Goode 1956; Jeffers & Scott 1953; Wynn 1968; Montgomerie 1977, 1984)

Genetics. *P. fragariae* is homothallic, and since its oospores can not be readily induced to germinate *in vitro*, inheritance studies on fungal traits seem not feasible. The ploidy level of *P. fragariae* is still unresolved, but since *Phytophthora* species are at least diploid, at least two alleles of a locus can be simultaneously present within an isolate.

(Bain & Demaree 1945; Duncan 1977; Brasier 1992)

Races. *P. fragariae* exists as races, which are per definition distinguished by differences in virulences on a set of differentiating strawberry genotypes. Various races have been distinguished in Canada, Germany, Japan, the US, and the UK. They may show some overlap because different differential sets have been used. Thus far, races have been denoted by a letter indicating the locality of origin, and a serial number. For instance, races A1, B1, and NS1 came from respectively the USA, Britain, and Nova Scotia (Canada).

RESISTANCE

Sources of resistance. Sources of race-specific resistance have been observed in various *Fragaria* species. The two sources most extensively and widely used are *F. x ananassa* Frith and its first and second generation descendants BK46, CC18, and Md683, and *F. x ananassa* Aberdeen, a cultivar with unknown parentage introduced in 1919. Their directed use was initiated in the late thirties and the early forties shortly after the recognition of their resistance and the recognition of the fungal origin of the disease. They have been brought together into cultivars with excellent horticultural characteristics in the USA and Canada. Resistances other than those from *F. x ananassa* have been consciously used in some initial crosses. Since their offspring had no commercial value, their directed use in breeding has frequently been discontinued. Resistances from *F.*

chiloensis and *F. ovalis* seem nevertheless to be accidentally present in some Oregon cultivars and selections.

A broad type of moderate resistance has been observed in Cambridge Favourite. This cultivar cropped well on naturally infested fields, although it usually became moderately diseased. The character of this resistance is a matter of dispute; some found it to be race-specific (Scott et al. 1975), while others considered it to be race-non specific (Gooding 1972, 1973; Kennedy et al 1986). Its mode of inheritance is also unresolved.

(Jeffers et al. 1940; Reid 1952; Jeffers & Scott 1953; Waldo 1953; Scott et al. 1962, 1975, 1984; Daubeny 1964; Daubeny & Pepin 1965; Jamieson & Nickerson 1989)

Tests for resistance. During the last forty years, an array of resistance tests has been developed. Regrettably they often gave rise to contradictory results; with some tests cultivars were classified as resistant, whereas with others they were classified as susceptible to the same fungal race. These inconsistencies hamper the identification of truly resistant genotypes, the differentiation of races, and eventually the elucidation of the genetics of resistance. Since the cause of these inconsistencies is poorly understood, it is unclear which tests are useful. The previous tests differ in type, amount, and administering of inoculum, incubation period, manner and frequency of watering, disease assessment, and account for escapes and incomplete resistance. In this thesis, the impact of some of these aspects will be examined.

Inheritance of resistance. Studies on the inheritance of resistance to red stele have been mainly conducted in conjunction with the breeding of new varieties. The observed segregation ratios did not seem to fit to simple genetic models for any of the resistance sources which have been identified. So it became commonly accepted that resistance to *P. fragariae* must be inherited polygenically. However, in other host-pathogen systems race specific resistance has been shown to be generally single-gene-based (Thompson & Burdon 1992). Since there are no obvious reasons why the genetic basis of resistance in strawberry should be of a totally different type from that in other crops, this contradiction gave rise to the hope of solving part the strawberry red stele puzzle.

The CPRO-DLO breeding program. CPRO-DLO initiated breeding for red stele resistance in 1968. It is nowadays the only still ongoing breeding program for red stele resistance in western Europe. Thus far, it produced a number of advanced selections combining resistance with a good horticultural value. However, this value remained still below that of the successful CPRO-DLO cultivar Elsanta, so none of them was released as cultivar. In this respect, the breeding for resistance is consistently at a disadvantage. Firstly, the horticultural value of the resistant crossing parents is less than that of the elite

susceptible cultivars. Secondly, just because of the inclusion of an additional trait (resistance) in the selection, the chance is reduced to find a genotype with all the desired characteristics.

Thus far, resistance tests at CPRO-DLO were performed in naturally infested fields. Although practically rewarding, this approach has the disadvantage of epistatic effects among the various sources of resistance; once one effective source had been incorporated, it masked the presence of other effective sources. This problem may be overcome by the use of artificial inoculations under controlled conditions with well defined isolates of the fungus.

Reasons for this thesis. Resistant cultivars can effectively combat the disease, and are thereby a natural alternative to soil fumigation. However, the breeding for resistance has thus far been complicated by the absence of a reliable resistance test, by a general lack of insight in the genetics of resistance, and by the occurring of races of the fungus. The research described in this thesis aimed to increase opportunities to create elite cultivars with red stele resistance by overcoming the former two complications.

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II. INHERITANCE OF RESISTANCE TO *PHYTOPHTHORA FRAGARIAE* HICKMAN IN STRAWBERRY

ABSTRACT

It has been found that complete resistance of the strawberry cultivars and selections Earliglow, Guardian, MdUS 2700, MdUS 2929, MdUS 3816, and Redchief to *Phytophthora fragariae* is inherited in a one-to-one ratio, suggesting that these genotypes have one major resistance gene effective to the races present in the test field. The moderate resistance of Cambridge Favourite was clearly recovered in about 50% of the selections descending from this cultivar. Since these selections were the result of preselection on horticultural characters on an uninfested field, no definite conclusions could be drawn as to the number of genes involved.

INTRODUCTION

The fungus *Phytophthora fragariae* Hickman is the causal agent of red core in strawberry. Complete and moderate resistance to *P. fragariae* are known to occur, but the genetics of which is still obscure. Complete resistance to *P. fragariae* is race-specific (Reid 1952; McKeen 1958; Hickman 1962; Montgomerie 1966; Converse 1970), due to which one may expect a limited number of major genes to be involved (Person 1959). However, thusfar no segregation ratios for resistance have been explained by the segregation of a limited number of genes (Scott et al. 1950; Stembridge & Scott 1959; Kronenberg et al. 1971; Melville et al. 1980). One of the factors which may limit the appearance of clear segregation ratios, is the octoploidy of strawberry cultivars. Senanayake & Bringhurst (1967) suggested the genomic formula of *F. x ananassa* Duch. octoploids to be AAA'A'BBBB, indicating that they behave like alloautopolyploids.

The Institute for Horticultural Plant Breeding (IVT) in the Netherlands has been concerned with the breeding for resistance to *P. fragariae* since 1968. This paper reviews the results obtained with segregating progenies as to inheritance of resistance. A distinction is made between complete resistance in a number of American cultivars and selections, and moderate resistance in the cultivar Cambridge Favourite (CF).

MATERIAL AND METHODS

Seedlings of 24 crosses were evaluated for resistance to *P. fragariae* a naturally infested field at Zundert during the 1977/1978 season. Their parentage is given in Table 1. During this same season 200-300 other seedlings of each of these crosses were selected for horticultural characters on an uninfested field at Elst, resulting in a total of 115 selections. These were evaluated for resistance to *P. fragariae* at Zundert during the following season. Seedlings and selections were planted in August.

Resistance of parental cultivars and selections. Parents were evaluated for resistance on the infested test field between 1974 and 1977. The American cultivars and selections Earliglow, Guardian, MdUS 2700, MdUS 2929, MdUS 3816, and Redchief were found to be completely resistant (R). Cambridge Favourite (CF) was susceptible (S), but showed a moderate level of resistance (S_{MR}). The IVT cultivars and breeding selections Induka, IVT 72138, IVT 72254, IVT 72343, Karina, Korona, Sivetta, and Tamella showed to be highly susceptible (S_{HS}) (unpublished results).

Races of the pathogen. It is not known which races of *P. fragariae* were present in the test field. Some of the differentials used by Montgomerie (1966) and Converse (1970) had been tested on this field. Of these, Cambridge Vigour, Juspa, and Redgauntlet were highly susceptible, whereas Siletz proved to be completely resistant (unpublished results).

Experimental design. With the 24 crosses on the infested field, a randomized complete block design was used with four blocks. Each block contained 24 plots consisting of 24 seedlings of the same progeny. The 115 selections were planted in a randomized fashion, each selection (clone) was represented by one plot of eight runner plants.

Observations. In April, half the number of plants were dug up and roots were evaluated for red core symptoms. The other half were used to evaluate the vegetative development in June, the data of which will not be referred to in this publication. The level of disease was scored on a scale of 1 - 9: score 1, all roots rotted, but evaluation cannot be done as it is uncertain whether this rot is caused by *P. fragariae*; score 2 = no symptoms; score 3, some roots show a red discoloration of the stele different from that caused by *P. fragariae*; score 4, less than 10% of the main roots show symptoms, score 5, 10-25%; score 6, 25-50%; score 7, 50-75%; score 8, 75-90%; and score 9, 90-100%. Plants rated 2 and 3 are considered symptomless; those rated 4 to 9 are considered susceptible. The two plants which rated 1 were excluded from the analysis.

RESULTS

Complete resistance. The percentage of symptomless seedlings of each cross is presented in Table 1. Most RxS crosses had significantly higher percentages of symptomless seedlings than the SxS crosses. The two exceptions are the RxS cross MdUS 2700 x Sivetta and the SxS cross Cambridge Favourite x Sivetta, which respectively have a relatively low and high percentage of symptomless seedlings.
All 18 RxS crosses had percentages of symptomless seedlings not significantly different from 50% according to Pearson's χ^2-test ($\alpha = 0.05$), except the just mentioned cross MdUS 2700 x Sivetta. In the SxS crosses, the average percentage of symptomless seedlings was 13%.

Numbers of selections without red core symptoms and total numbers of selections derived from the uninfested field at Elst are presented in Table 2. Of the 79 selections from RxS crosses, 39 (=49%) showed to be completely resistant to *P. fragariae*.

Table 1. Percentages[a] of symptomless plants in 24 crosses during the season of 1977/1978.

		Susceptible parents (S)								
		Highly Susceptible (S_{HS})								Moderately resistant (S_{MR})
		Induka	Karina	Korona	Sivetta	Tamella	IVT 72138[b]	IVT 72254[b]	IVT 72343[b]	Cambridge Favourite (CF)
Completely Resistant parents (R)	Earliglow		51 abc						63 a	
	Guardian	63 a[c]			58 ab	49 abc		55 ab		44 abcd
	Redchief	60 ab			54 ab			52 ab		
	MdUS 2700				33 cde			46 abcd		42 bcd
	MdUS 2929				59 ab		56 ab			
	MdUS 3816	48 abc			46 abcd					56 ab
Susceptible parent (S_{MR})	Cambridge Favourite (CF)	4 g		13 fg	29 def	13 fg	15 efg	4 g		

a: Each percentage is the mean of four replications.
b: IVT 72318 = Tamella x Induka, IVT 72254 = Tamella x Redgauntlet, IVT 72343 = Induka x Sivetta
c: Different letters indicate a significant difference according to a two-sided *t*-test (α=0.05, S.E.D.= 0.018).

Moderate resistance. To examine the inheritance of the moderate resistance of CF, two groups of susceptible seedlings were distinguished: (1) susceptible seedlings descending from CF, and (2) susceptible seedlings descending from crosses between a resistant (R) and a highly susceptible (S_{HS}) parent. The first group showed a higher percentage of

Table 2. Number of selections without red core symptoms (numerator), and total number of selections (denominator) selected at the uninfected field at Elst.

		Susceptible parents (S)								
		Highly Susceptible (S_{HS})							Moderately resistant (S_{MR})	Σ
		Induka	Korona	Sivetta	Tamella	IVT 72138	IVT 72254	Σ	Cambridge Favourite (CF)	
Completely resistant parents (R)	Guardian			4/5	2/3		1/5	7/13	3/5	10/18
	Redchief	1/3		6/7			2/3	9/13		9/13
	MdUS 2700			2/5			3/6	5/11	2/8	7/19
	MdUS 2929			1/5		3/8		4/13		4/13
	MdUS 3816	1/2		4/6		0/1		5/9	4/7	9/16
Σ		2/5		17/28	2/3	3/9	6/14	30/59	9/20	39/79
Susceptible parent (S_{MR})	Cambridge Favourite	0/4	0/7	0/8	0/6	0/6	0/5	0/36		

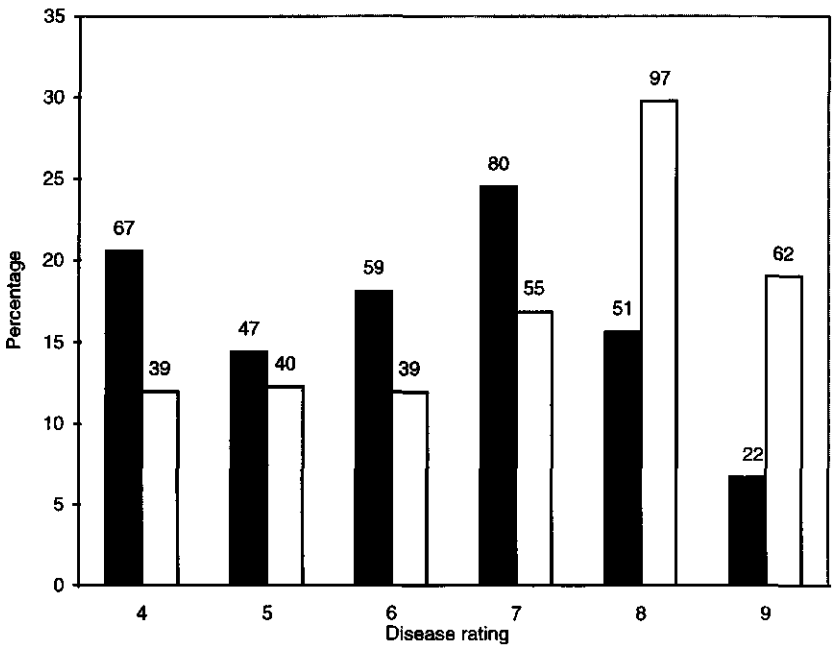

Figure 1. Frequency distribution (in %) of disease rates of 326 and 322 susceptible seedlings of which Cambridge Favourite respectively was (■) or was not (□) one of the parents. Numbers above bars indicate absolute numbers of seedlings corresponding with percentages. The meaning of disease rates is given under Material and Methods.

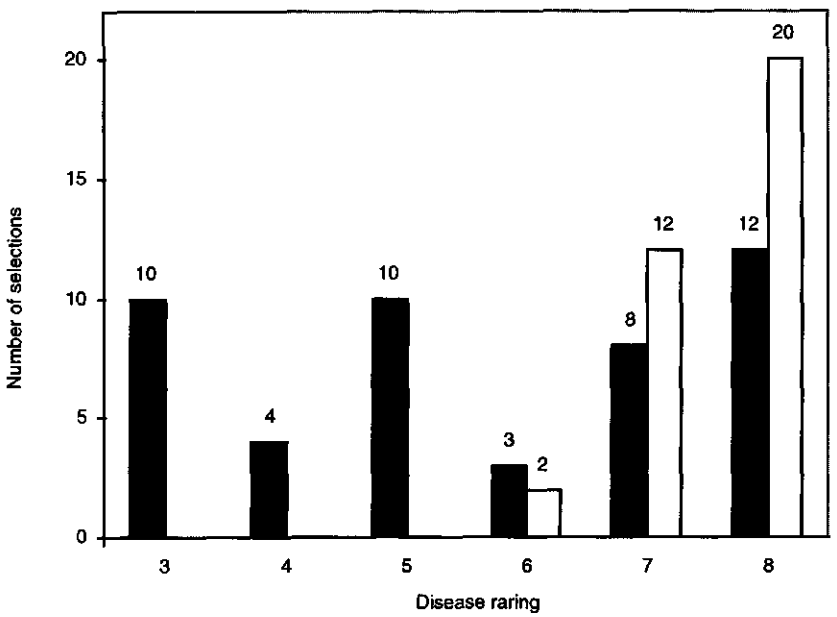

Figure 2. Distribution of mean disease rates of 47 susceptible selections with Cambridge Favourite being a parent (■), and of 29 susceptible selections with Cambridge Favourite not being a parent (□). The meaning of disease rates is given under Material and Methods.

seedlings with low disease levels (Figure 1). This difference was significant according to the Kolmogorov-Smirnov two-sample test (α=0.001, Siegel 1956), which shows that the CF resistance is genetically determined.

The number of symptomless selections as well as the total number of selections of each cross is presented in Table 2. Of 59 selections from the RxS$_{Hs}$ crosses, 30 were completely resistant and 29 were highly susceptible. Of the 56 selections from the RxS$_{Mr}$ and SxS$_{Pr}$ crosses, 9 showed to be completely resistant. Of the remaining 47 susceptible selections, 24 (=51%) were less infected than any of the 29 susceptible selections from the RxS$_{Hs}$ crosses and were classified as moderately resistant.

DISCUSSION

Complete resistance. It was shown that 17 out of the 18 RxS crosses had percentages of symptomless seedlings not significantly different from 50%. This percentage can be explained by a monogenic inheritance with one + allele for resistance. As one divergent percentage out of 20 is allowed to occur when testing at the 95% confidence level, this result suggests that the resistant parents contain only one + allele effective to the races

present in the test field.

Clear segregation ratios had not been found in earlier inheritance studies (Scott et al. 1950; Stembridge & Scott 1959; Kronenberg 1971; Melville et al. 1980). This may have several causes, e.g. contamination of the inoculum by unidentified races, and/or the use of genotype-race combinations in which (part of) the resistance genes behave complementary, and/or differences among cultivars in number of + alleles at individual loci. In all cases segregation of individual resistance genes will probably be masked. It may be interesting to investigate whether cultivar-race interactions may be of help to clarify the genetic basis of resistance since in other host-pathogen systems this approach has let to the identification of individual resistance genes of the host and avirulence genes of the pathogen (Flor 1956, 1971; Parlevliet & Zadoks 1977).

The observed percentage of completely resistant selections of the RxS crosses are well in agreement with those for the seedlings, all being around 50%. This indicates an absence of linkage between the resistance gene and the genes affecting the horticultural characters selected for. Finally, it was expected that symptomless plants would be absent in SxS crosses. However, on average 13% of these seedlings showed no symptoms of the disease (Table 1). Whatever the cause of the occurrence of this percentage may be, it has no influence on the evidence that the complete resistance of the examined resistant American parents is inherited monogenically.

Moderate resistance. CF has a high level of moderate resistance according to Gooding (1972) and to the authors experience (unpublished results). Figure 1 shows that this cultivar passed its resistance in a quantitatively detectable way on to its progeny. However, from the results no final conclusions can be drawn with regard to the number of genes involved, and that for several reasons. For instance, the frequency distribution of the susceptible seedlings from CF does not indicate the presence of different classes (Figure 1). Besides, a considerable fraction of the highly susceptible seedlings may have escaped from severe infection since a considerable part of the RxS_{HS} crosses was also only moderately diseased. It is nevertheless very suggestive that about 50% of the susceptible selections derived from CF was less infected than any of the selections from RxS_{HS} crosses since this percentage is expected in case of a monogenic inheritance (with one + allele) and absence of linkage between this gene for moderate resistance and the genes for horticultural characters. This result further indicates that the completely resistant (American) parents contain no genes for moderate resistance to the *P. fragariae* races present in the test field.

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III. A QUANTITATIVE CLASSIFICATION METHOD FOR ASSESSING RESISTANCE TO *PHYTOPHTHORA FRAGARIAE*

ABSTRACT

Race specific resistance to red core (red stele) root rot, caused by *Phytophthora fragariae* var. *fragariae*, is known to occur in the cultivated strawberry (*Fragaria x ananassa* Duch.), but frequently this resistance does not completely prevent infection. We therefore developed a classification method which distinguishes moderate and complete resistance. It accounts for differences in the aggressiveness of isolates and in the appropriateness of experimental conditions for infection. This method is based on the microscopically assessed disease severity of a tested strawberry genotype relative to that of an universally susceptible reference cultivar. If the tested genotype is significantly ($P \leq 0.01$) less diseased, it is considered to possess resistance. Data from 18 genotype-isolate combinations, including five genotypes (Blakemore, Md683, Redgauntlet, Del Norte, Yaquina A) and four North American isolates (A2, A4, A6, and A10) demonstrated the validity of our classification scheme.

INTRODUCTION

Phytophthora fragariae Hickman var. *fragariae* (Wilcox et al. 1993), is a soilborne fungus which causes red core (red stele) root rot in strawberry (Hickman 1940). Many cultivars have been tested for race specific resistance (Scott et al. 1950. 1975; McKeen 1958; Pepin & Daubeny 1964, 1966, Montgomerie 1966; Converse 1970; Kennedy et al. 1986; Maas et al. 1989; Milholland et al. 1989; Nickerson & Murray 1993), but investigators often reached different conclusions. These inconsistencies hamper differentiation of cultivars and isolates, elucidation of the genetics of resistance, and breeding for resistance (Van de Weg 1989). Part of the inconsistencies may be due to differences between classification methods used to discriminate between resistance and susceptibility. All methods applied thus far use a single, more or less arbitrarily chosen disease severity threshold for classification for all isolates and all experimental conditions. In this paper we propose a classification method which relies on a flexible threshold based on the disease severity of a highly and universally susceptible reference cultivar. Results obtained with this approach are compared with published results of a set of strawberry genotypes and North American isolates.

MATERIAL AND METHODS

Plant material. The study included strawberry genotypes of *F. x ananassa* (Blakemore, Redgauntlet and Md683) and *F. chiloensis* (L.) Duch. (Del Norte and Yaquina A). Blakemore, Md683, Del Norte and Yaquina A are part of the differential set used to differentiate the North American isolates A1 to A10 (Converse 1970). Md683 originated from the USDA strawberry breeding program at Beltsville, MD (Scott et al. 1950). Blake more is highly susceptible to all these isolates while the other genotypes are resistant to some of them (Converse 1970; Milholland et al. 1989). Stock plants of Blakemore, Del Norte and Md683 were kindly supplied by R.D. Milholland (North Carolina State Univer sity, Raleigh, NC), and those of Yaquina A were supplied by K.E. Hummer (USDA National Germplasm Repository Corvallis, OR). Plants of Redgauntlet came from the CPRO-DLO strawberry collection. Stock plants were grown in a greenhouse with a photoperiod of 16 h (supplemented with incandescent light) and in which the temperature varied with a minimum of 18°C during the day and 13°C during the night. For inoculation experiments, young, free-hanging runner plants were excised, planted in an autoclaved (4 h, 120°C) mixture of peat-based compost and sand (1:1 by volume) and rooted for 18 - 21 days under intermittent mist in a greenhouse with a similar temperature regime as above. After rooting, plants were carefully lifted and the compost/sand mixture removed by thoroughly rinsing the roots in tap water.

Isolates. The study included the USA isolates A2, A4, A6 and A10, which were differentiated by Converse et al. (Converse & Scott 1962; Converse 1970). Isolates A2 and A10 were supplied by R.D. Milholland, and A4 and A6 by J.L. Maas (USDA, Beltsville, MD). Stock cultures were maintained at 8°C on modified kidney bean agar (KBA) (Maas 1972) prepared by adding 35 g of finely ground light-red kidney beans (Berger and Company, LaSalle, CO), to 500 ml of distilled water. Technical Agar (17 g) (Code L13; Unipath LTD, Basingstoke, UK) was added to another 500 ml of water. Both solutions were autoclaved for 20 minutes at 120°C and mixed just before dispersing aliquots of $\pm$ 20 ml into plastic petri dishes (90 x 15 mm).

Inoculation procedures. Inoculation procedures were based on those of Wynn (1968) and Maas (1970). For inoculum production, three to five pieces (1-4 mm^2) of colonized KBA from stock cultures were transferred to each petri dish of a series containing fresh KBA. After an incubation period of 18 days at 18 $\pm$ 1°C in the dark, during which time colonies had not yet grown to the edge of the dish (Wynn 1968; Mussel & Fay 1973), excised colonies, consisting of mycelium and the agar beneath it, were put into a blender with distilled ice water (1 g culture: 1 ml water) and comminuted twice for 2 s (19 x 10^3

rpm). The resulting slurry was transferred to a cooled mortar which was kept in ice during the entire inoculation procedure. Plants were inoculated by dipping their roots into the slurry, and then planted in plastic pots (8.5 x 8.5 x 9.0 cm) containing an autoclaved compost/sand mixture (see plant material).

Experimental conditions. After inoculation, plants were placed in a walk-in growth chamber with laminar air-flow under 16 h fluorescent light (TLD 58W/84, Philips). Light intensity decreased from 291 $\pm$ 17 μmol.m^{-2}.s^{-1} at the onset of this research to 200 $\pm$ 18 μmol.m^{-2}.s^{-1} at the end due to aging of the lamps. The soil temperature fluctuated between 15 $\pm$ 1°C during the light period and 10 $\pm$ 1°C during the dark period. Inoculated plants stood in a shallow layer of water (2 to 7 mm) during the first three to four days after inoculation (Hickman 1940). Thereafter, plants were watered daily with 50 ml of cool (circa 12°C) tap water under conditions of free drainage.

Disease assessment. During the fourth week (22-28 days) after inoculation, plants were removed from the pots and roots rinsed free of soil with tap water. The length of each main (adventitious) root was measured, discarding purpled root ends as these never contained oospores and are not symptoms of the disease. Main roots with any external discoloration were scanned microscopically for the presence of oogonia or oospores. Here fore the entire length of discoloured tissue of each root was excised, together with some adjacent symptomless tissue, squashmounted on a microscope slide and examined at 100x magnification at high light intensity (12V 25W halogen lamp). Roots without external symptoms were cut longitudinally; those which showed internal discoloration were also examined microscopically. To assess the length of colonized tissue, the microscope slide was moved so that the most apical and distal oogonium, or oospore if oogonia were not present, were each placed in the centre of a microscopic field and the light beam was focused to a pin-point. The resulting light spots were marked on the slide and the distance between these marks was measured in millimetres.

The percentage invaded root tissue (PIRT) of a plant was calculated by expressing the total length of root tissue that contained oospores or oogonia as a percentage of the total length of the main roots. Final PIRTs of genotypes are the mean of the averages of the experiments calculated on the probit scale (see below).

Experimental design. Each genotype-isolate combination was tested using four plants which were equally divided over two mutually independent experiments. Combinations were examined with an additional number of two to four plants if the initial experiments gave variable results (example: Del Norte-A6) or if an isolate of the fungus was only slightly aggressive (A10). Each experiment included four plants of Blakemore and two

plants of at least one of the other genotypes. Only four plants of Yaquina A were tested per experiment to account for its relative low number of roots at the time of inoculation. Yaquina A was not tested against all isolates as it became available only later during this study.

Statistical analyses. Regression analysis of PIRTs was performed on a probit-transformed scale (McCullagh & Nelder 1983) using a split-plot design with experiments as main plots and genotypes and isolates as factors, of which isolates were confounded with main plots. Before transformation, values lower than 0.45% and higher than 99.55% were replaced by 0.45% and 99.55% respectively. Significance of pairwise differences between genotypes and Blakemore were examined by the one sided *t*-test (Mead et al. 1993). Threshold-PIRTs were calculated according to the formula $Y = X - t_{\alpha} * SED$; in which
Y is the probit of the PIRT for the threshold;
X is the probit of the PIRT for Blakemore;
t_{α} is the *t*-value for the selected confidence level;
SED is the standard error of differences.
Next, *Y*-probits were transformed back to percentages.
The statistical package GENSTAT (Genstat 5 committee 1987) was used for analyses.

RESULTS

Roots of Blakemore and Del Norte were the most invaded of all genotypes with all the tested isolates, while Md683 and Yaquina A were not or only slightly invaded (Table 1). Genotypes were tested simultaneously with Blakemore thus enabling quantitative comparisons between their PIRT and that of Blakemore. For each genotype, its threshold PIRT was calculated at the 99% confidence level (Table 1) based on the disease reaction of Blakemore. Genotypes are classified as resistant if their PIRT remained below and as susceptible if their PIRT exceeded this threshold (Table 2).

DISCUSSION

We propose a classification method which accounts for moderate as well as complete resistance. The method classifies genotypes as resistant if they are significantly less diseased than the reference cultivar Blakemore, which is highly susceptible to all North American isolates of *P. fragariae* var. *fragariae* (McKeen 1958; Converse & Scott 1962; Converse 1967, 1970; Nickerson & Murray 1993). As a result, resistant genotypes may

Table 1. Average percentages of invaded root tissue (PIRTs) of four tested strawberry genotypes (TG) inoculated with four races of *P. fragariae* var. *fragariae*, PIRTs of the reference cultivar Blakemore (Bl), and relative disease thresholds (Th)[a]

Genotype	Isolate											
	A2			A4			A6			A10		
	TG	Bl	Th	TG	Bl	Th	TG	Bl	Th	TG	Bl	Th
Md683	2	37	11[b]	5	63	30	1	45	16	0	11	3
Redgauntlet	0	37	11[b]	0	63	30	30	45	16	1	11	3
Del Norte	33	37	11[b]	58	63	30	13	31	12	7	11	3
Yaquina A	nt[c]			0	25	14	nt			0[d]	10	2

a: for the 99% confidence level.

b: Genotype-race combinations which have the same threshold were examined simultaneously throughout the research. Each pair of PIRTs of a tested genotype and Blakemore originates from the same series of experiments.

c: not tested.

d: one plant out of eight showed some infected root tips, giving a PIRT of less than 0.1%.

Table 2. Proposed classification of genotypes as resistant (-) or susceptible (+) to single races of *P. fragariae* var. *fragariae*. The *t*-value for the significance of the difference between the PIRT of the test genotype and that of Blakemore is given in parentheses[a].

Cultivar	Isolate							
	A2		A4		A6		A10	
Blakemore	+		+		+		+	
Md683	-	(-4.7)	-	(-5.4)	-	(-5.6)	-	(-4.5)
Redgauntlet	-	(-5.1)	-	(-7.9)	+	(-1.1)	-	(-3.5)
Del Norte	+	(-0.3)	+	(-0.4)	+	(-2.2)	+	(-0.8)
Yaquina A	nt[b]		-	(-7.4)	nt		-	(-4.4)

a: Critical *t*-values for the 95%, 99%, and 99.5% confidence level are respectively -1.7, -2.4, and -2.6 (101 degrees of freedom).

b: not tested

still be moderately diseased. This approach is in accordance with the quantitative character of resistance (Anonymous 1985), but differs from earlier methods which were all directed at immunity or at a resistance level near to immunity. Using the disease reaction of the reference cultivar, this approach accounts for differences in the aggressiveness of isolates and the appropriateness of experimental conditions for infection and invasion. As a statistical test is included, it also takes into account experimental variation.

Thresholds. The flexible threshold-PIRTs used to differentiate between resistance and susceptibility (Table 1) reflect the features of the classification method. The thresholds for A10 were considerably lower than those for the other isolates because of the lower levels of invasion of Blakemore by A10 (Table 1). The reproducible low level of disease of the combination Blakemore-A10 demonstrates that this isolate is less aggressive than the other isolates tested.
Also, the combinations Md683-A4 and Yaquina A-A4 were tested in different series of experiments. The higher PIRT of Blakemore in the Md683 series (Table 1) reflects superior conditions for infection and resulted in a higher threshold. Thresholds also will increase with decreasing confidence levels, decreasing experimental variation and with larger numbers of plants tested per genotype.

The flexibility of this threshold contrasts with the fixed thresholds of earlier classification methods, which classified a cultivar as resistant either if it never showed any infection (Pepin & Daubeny 1964, 1966; Montgomerie 1966), if invasion was confined to the root tips (Converse & Scott 1962; Converse 1967, 1970), if invasion was limited to 3% or less of the total root length (Nickerson & Murray 1993) or if it remained below a certain level of a disease severity index (Milholland et al. 1989). These thresholds are more or less equivalent to fixed PIRTs of 0.01%, 3%, 3%, and 5%-10%, respectively.

Moderate resistance. Most of the thresholds are quite high, compared to those applied in the literature, and allow a moderate level of invasion of resistant genotypes. This means that the present approach takes into full account moderate levels of resistance. To identify still lower levels of resistance, more plants would need to be tested. For instance, if the observed differences in PIRTs between Del Norte and Blakemore reflect actual differences in resistance, then approximately 1600, 800, 280 and 40 plants would be needed of both genotypes to test against respectively A2, A4, A10, and A6 (α=0.01, ß=0.01). Obviously, the investment needed to test such high numbers is too great to detect such low levels of resistance. At this time, no information is available on the correlation between levels of resistance as assessed in the laboratory and as expressed in the field.

Escapes. A problem with any classification method is that highly susceptible plants may escape infection. For our classification it means that if plants of a susceptible test genotype escape, this genotype will more likely be classified as resistant. By contrast, if plants of Blakemore escape, genotypes with moderate levels of resistance will more likely be classified as susceptible. Indications for the presence of escapes come from variable results among the individual plants of a genotype-isolate combination. Additional tests

would be needed either to decrease the impact of divergent results on the average PIRT or to decide which of the inconsistent results is reproducible and which should be ignored. For instance, Del Norte-A6 was tested in three experiments due to variable results between the first two. The results of the third experiment were consistent with the first, Del Norte being as severely invaded as Blakemore. In the second experiment, Del Norte probably escaped from severe infection, its two plants having PIRTs of 0% and 6.5% while Blakemore rated 9%, 16%, 18% and 45%. These former ratings greatly decreased the average PIRT of Del Norte. Consequently it could be classified as susceptible at the 99% level (Tables 1 and 2) and as resistant at the 95% level (Table 2). When the deviating ratings were excluded from the analysis, Del Norte was classified susceptible at the 95% as well as at the 90% confidence level (t-value = 1.0, 100df).

Number of replicates. The number of plants to be tested for reliable classifications greatly depends on the frequency of escapes. Of the plants of the compatible combinations 15% escaped severe infection, 7% because they showed no infection and 8% because they were only slightly diseased. Maas (1970) also found 7% symptomless plants when testing single isolates (A2 - A9) on Blakemore at 20% to 30% lower inoculum densities.
The chance that a single susceptible genotype is classified as resistant to a isolate because all four plants tested escaped from severe infection is 0.05% ($= 0.15^4 \times 100\%$) under a binomial distribution. The chance that all classifications are valid when a series of six susceptible genotypes is examined, can be estimated as 99.7% ($= (1 - 0.0005)^6 \times 100\%$). We therefore consider four plants to be sufficient for reliable classifications, given that the individual plants of resistant genotypes gave consistent results. Higher numbers of plants would be required for the assessment of moderate levels of resistance, as discussed above.

Statistical test. The efficacy of the statistical test can be demonstrated by its thresholds, as shown, or by the t-values for the significance of differences. Thresholds are very informative at the plant level. However, t-values are more informative for statistical analysis. They can be directly interpreted at any desired confidence level and they can be more clearly structured in case of unbalanced experiments. In this study, t-values lower than -2.4 indicated that a genotype was significantly less invaded than Blakemore (99% confidence level)(Table 2).

Microscopical observations. Infection of strawberry by *Phytophthora fragariae* var. *fragariae* is generally assessed macroscopically on the basis of the extent of reddening of the stele. However, this red colour does not necessarily indicate the actual presence of the fungus (Hickman 1940; Otterbacher et al. 1969; George & Milholland 1986) and the

brownish colour of dead roots may mask reddened steles (Hickman 1940; Jeffers & Scott 1953). To obtain a more reliable estimate of the disease severity, we assessed the extent to which oogonia and oospores were present.

Comparison with earlier classifications. Fifteen classifications are in agreement with the literature, and three are divergent from at least one earlier report (Md683-A2, Md683-A6, Yaquina A-A10) (Converse & Scott 1962; Montgomerie 1966; Pepin & Daubeny 1966; Converse 1970; Scott et al. 1975; Milholland et al. 1989; Nickerson & Murray 1993). These divergences can be explained by differences in the applied disease thresholds and experimental parameters. Converse and co-workers (Converse & Scott 1962; Converse 1970) classified genotypes on the basis of the most severely invaded roots of the most severely invaded plant out of a series. Genotypes were classified as susceptible if invasion extended beyond the root tip. Md683 was classified as susceptible for A2 as some roots were invaded up to 25% of their length. We think that focusing on the most severely invaded roots may result in a distorted impression of the susceptibility of a genotype. The present classification is therefore based on the average level of invasion of all main roots in a series of plants. Also in this research Md683 had some roots with red steles up to 25% of their length, but the average degree of invasion was significantly lower than that of Blakemore. The resistant reaction of Md683 is in agreement with results of Scott et al. (1975) and was also confirmed in a third experiment, which led to a final PIRT of 1% for Md683, 53% for Blakemore, and 28% for the threshold, and a *t*-value of -8.3 (data not shown).

Similarly, Converse (1970) classified Yaquina A as susceptible for A10 as some roots were invaded to the crown (rating 2 on a scale of 1 (dead) to 9 (no symptoms)). However, Converses' (1970) average rating for Yaquina A was 7, indicating that various plants were not or only slightly diseased, while Blakemore had an average rating of 2, indicating that all plants were severely infected. Our data (Table 1) and those of Milholland et al. (1989) confirm the extremely low susceptibility of Yaquina A. We therefore conclude that Md683 and Yaquina A must be classified as resistant, although individual roots may, under some conditions, be considerably invaded. The present classifications affect the original key to the USA isolates (Converse 1970). This key can no longer differentiate among A2, A4 and A10 since A2 and A4 were originally differentiated by the presumed susceptibility of Md683 to A2, while A4 and A10 were differentiated by the presumed susceptibility of Yaquina A to A10.

Milholland et al. (1989) rated genotypes on the basis of a disease severity index comprising the percentage of infected root tips and the average number of oospores per

root tip. Genotypes with an index lower than 1 were classified as resistant. Md683 was considered to be susceptible to A6, having an index of 7. However, this index differed so vastly from the one of the highly susceptible genotype Tennessee Beauty (index of 65) that the experimental error as the sole cause is highly unlikely. It is therefore that Md683 is resistant to A6.

Montgomerie (1966) and Pepin & Daubeny (1964, 1966) classified a cultivar as susceptible as soon as a single oospore was observed. It needs no explanation that in this way many cultivars will be classified as susceptible which are resistant according to the methods discussed above. Indeed all differences in classifications concern cultivars which are presently classified as resistant.

Relevance of inoculation procedures and experimental conditions. Different inoculation procedures have been used by various investigators: a suspension of encysted zoospores sprayed onto bare roots (Milholland et al. 1989), a zoospore suspension poured over the surface of the compost of potted plants (Nickerson & Murray 1993), a suspension of zoospores and mycelium poured into furrows near the roots of established plants (Converse 1970), and roots dipped into a slurry of macerated mycelium (this research). Also, different experimental conditions were applied, including differences in watering regimes and incubation periods. While not discussing the advantages and disadvantages of each approach, we emphasize that all methods lead to consistent results when our classification method is applied to the data, at least for the cultivar-isolate combinations examined in this research. This shows that the way in which disease severity data are interpreted is the most essential part of classification.

In conclusion. The unique feature of the classification method is the comparison of the disease severity of each strawberry genotype with that of a highly susceptible reference cultivar. This approach gave consistent results when applied to the present data and to data reported in the literature. We therefore consider this an improved method for reliably assessing the resistance of strawberry genotypes and the virulence of isolates of *P. fragariae* var. *fragariae*.

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IV. ASSESSMENT OF THE RESISTANCE TO *PHYTOPHTHORA FRAGARIAE* VAR. *FRAGARIAE* OF THE USA AND CANADIAN DIFFERENTIAL SERIES OF STRAWBERRY GENOTYPES.

ABSTRACT

The resistance of the USA and Canadian differential series of strawberry (*Fragaria* spp.) genotypes was assessed against nine USA isolates (A1-A4, A6-A10) and three Canadian isolates (NS2-NS4) of *Phytophthora fragariae* var. *fragariae*. Also, the resistance of part of the UK series was examined. Of the 157 host genotype-isolate combinations tested, 13 were classified differently from earlier reports. These differences can be explained by the existence of different clones under the same cultivar name (Aberdeen and Perle de Prague), the substitution of Aberdeen by Sparkle, which differ in their resistance, and by the application of different criteria for resistance. Here, incomplete resistance was considered as resistance.

The discrimination of the USA differential series can be improved by including the UK differential Perle de Prague and the Canadian differential Sparkle. The Canadian series can be improved by including Perle de Prague and the US differential Aberdeen. This leads to one unified differential set for North America.

The results also allow the proposition of a formal gene-for-gene model which, in turn, provides for a universal differential series for this pathosystem.

INTRODUCTION

Phytophthora fragariae (Hickman 1940) var. *fragariae* (Wilcox et al. 1993) is a soil-borne fungus, causing red core (red stele) root rot in strawberry and of which various races exist. Differential series have been developed in the USA (Converse & Scott 1962; Converse 1967, 1970), the UK (Montgomery 1966; Kennedy et al. 1986; Kennedy & Duncan 1988) and Canada (Nickerson & Murray 1993) to distinguish isolates and potential sources of resistance. Since the validity of these series has been a matter of dispute, investigators have frequently reached different conclusions on the resistance of strawberry genotypes and the virulence of fungal isolates (Montgomery 1966; Kennedy et al. 1986; Kennedy & Duncan 1988; Milholland et al. 1989; Van de Weg et al. 1996). These different results impede not only the design of effective differential series, but also the proposition of a reliable gene-for-gene model to clarify the genetics of resistance in strawberry and the genetics of avirulence in the fungus (Van de Weg 1989a, 1989b).

Recently, Van de Weg et al. (1996) proposed a quantitative classification method for resistance which produced consistent results when applied to their own data and other data. In the present paper, we report an evaluation of the resistance of the USA and Canadian differential series and part of the UK one in an attempt to develop a universal differential series of strawberry genotypes.

MATERIAL AND METHODS

Plant material. The strawberry genotypes included in this study are listed in Table 3, as are the differential series in which they occur. Del Norte, Yaquina A, and Yaquina B are clones of *Fragaria chiloensis* (L.) Duch., all other genotypes are of *F. x ananassa*. Our sources of Del Norte, Yaquina A, Blakemore, Md683, and Redgauntlet have been described previously (Van de Weg et al. 1996). Yaquina B was obtained from K. Trajkovski (Swedish University of Agricultural Sciences, Balsgård, Sweden), Aberdeen-NCSU, Climax and Sparkle from R.D. Milholland (North Carolina State University, Raleigh, NC, USA), Stelemaster from N.L. Nickerson (Research Station Kentville, Nova Scotia, Canada), and Aberdeen-SCRI, Auchincruive No.6 (Auch.6), and Perle de Prague (Perle) from D.M. Kennedy (Scottish Crop Research Institute, Invergowrie, Scotland). Senga Sengana and Siletz came from the CPRO-DLO strawberry collection.

Isolates. The study included all 10 isolates of the USA test series (A1-A10) (Converse 1970) and three of the four isolates of the Canadian test series (NS2, NS3, and NS4)(Nickerson & Murray 1993), all of which originated from single zoöspores (Converse & Scott 1962; Converse 1967, 1970; Nickerson & Murray 1993). The USA isolates were obtained from J.L. Maas (USDA, Beltsville, MD, USA) except A2 and A10, which were supplied by R.D. Milholland. The Canadian isolates came from N.L. Nickerson and are subcultures of her cultures 25, 34, and 35 (Nickerson & Murray 1993).

Experimental procedures. The method of inoculum production, inoculation, disease assessment, and of classifying genotypes as resistant or susceptible have been described previously, as have the experimental conditions, the experimental design and the statistical procedures (Van de Weg et al. 1996). Briefly, young runner plants were inoculated by dipping their roots into a suspension of macerated fungal mycelium. Next, they were grown in a walk-in growth chamber under conditions conducive to the disease. After 4 weeks, plants were lifted to assess microscopically the percentage of invaded root tissue (PIRT). Genotypes were classified as resistant if their average PIRT was significantly less

than the PIRT of the universally susceptible cultivar Blakemore as assessed in the same tests (one sided *t*-test, 99% confidence level). Each genotype-isolate combination was generally tested using four plants split equally between two independent experiments; a further two to four plants were examined if the initial experiments gave variable results or if an isolate was only slightly aggressive (A5 & A10).

RESULTS

The observed PIRTs of each genotype are given in Table 1, in which genotypes are arranged according to the existing differential series. The *t*-values for the difference between the PIRT of the tested genotypes and cultivar Blakemore are presented in Table 2. Table 3 shows the conclusions on the (in)compatibility of genotype-isolate interactions. Genotypes with *t*-values lower than -2.3 were significantly (99% confidence level) less diseased than Blakemore, and were consequently classified as resistant. For example, Md683 is considered to be resistant against A1 as its PIRT of 1.0 was significantly less than Blakemore's PIRT of 28 (*t*-value -6.1). Data on A5 are not presented as all PIRTs were extremely low, including those of cultivar Blakemore. Stelemaster, Yaquina A, and Auch.6 were not tested against all isolates as they became available only later during this study.

Senga Sengana was susceptible to all isolates and may therefore be used as a universally susceptible differential, like cultivar Blakemore. Sparkle, Climax, and Redgauntlet each had a similar resistance pattern, as did Md683, Aberdeen-NCSU, and Auch.6. The same is true for Yaquina A and Yaquina B and also for Stelemaster and Siletz. The two accessions of Aberdeen differed in their resistance. Aberdeen and Md683 are known to have a different resistance (Scott et al. 1950, 1953, 1984; Converse et al. 1958; Converse & Scott 1962; Daubeny 1964; Converse 1967). Since Aberdeen-NCSU had the same resistance pattern as Md683, Aberdeen-NCSU cannot be true to type and will not be further considered.

Of the 157 cultivar-isolate combinations examined, 33 are reported for the first time, while 58 were previously examined when developing the USA differential series (Converse & Scott 1962; Converse 1966, 1970), 15 when developing the Canadian differential series (Nickerson & Murray 1993), and 86 in the research of Milholland et al. (1989). Six classifications differ from the USA observations (Md683-A2, Md683-A8, Aberdeen-A6, Aberdeen-A8, Yaquina A-A9, and Yaquina A-A10), one from the Canadian observations (Sparkle-NS4), and six from earlier reports on British differentials (Siletz-A6, Perle-A1, Perle-A3, Perle-A6, Perle-A7, and Perle-A8).

Table 1. Average percentage of invaded root tissue (PIRT) of 14 strawberry genotypes (indicated by GR) by 12 isolates of *P. fragariae* var. *fragariae* and the respective PIRT of the reference genotype Blakemore (Bm).

Genotype	Isolate											
	A1[a]		A2		A3		A4		A6		A7	
	GR	Bm[b]	GR	Bm	GR	Bm	GR	Bm	GR	Bm	GR	Bm
Md683	1	28	1	50	t	29	5	62	2	45	30	42
Aberdeen-NCSU	1	26	t[d]	37	1	31	1	63	0	45	32	50
Aberdeen-SCRI	1	26	0	37	20	22	0	63	t	45	t	42
Sparkle	19	28	t	37	34	31	0	63	22	45	t	50
Del Norte	0	28	33	37	1	22	58	63	25	36	3	42
Stelemaster	[c]								16	36	1	22
Yaquina A	0	24			0	25	0	33	0	11	1	29
Perle	0	26	t	51	t	22	1	62	t	45	47	48
Climax	19	26	1	37	14	22	1	63	35	45	t	48
Redgauntlet	28	26	0	37	33	31	0	63	30	45	t	48
Siletz	1	28	1	37	t	22	0	63	1	45	t	50
Auch.6									14	45	39	22
Yaquina B	0	28	0	37	t	22	0	63	0	45	t	48
S.Sengana	25	35	18	13	44	39	35	63	28	45	48	50

Table 1 Continued

Genotype	Isolate											
	A8		A9		A10		NS2		NS3		NS4	
	GR	Bm	GR	Bm	GR	Bm	GR	Bm	GR	Bm	GR	Bm
Md683	38	25	3	40	0	11	2	35	21	36	34	33
Aberdeen-NCSU	14	26	2	51	1	11	1	35			30	35
Aberdeen-SCRI	1	26	t	51	t	11	21	35	0	32	22	33
Sparkle	35	26	t	44	1	11	58	35	t	36	22	31
Del Norte	2	26	15	40	6	11	24	35	t	36	1	33
Stelemaster	27	16					2	70	8	70	27	31
Yaquina A			t	13	t	10			0	43		
Perle	4	25	5	51	t	11	t	35	15	36	32	33
Climax	60	26	1	51	1	11	43	35	1	36	41	33
Redgauntlet	29	26	t	51	2	11	40	35	1	32	65	33
Siletz	35	26	t	51	1	11	1	35			34	33
Auch.6	10	16					1	70			11	25
Yaquina B	0	26	1	51	1	11	1	35	t	32	0	33
S.Sengana			57	50			43	38	50	32	31	33

a: A1-A4, A6-A10 are part of the USA pathogen differential series (Converse 1970), and NS2-NS4 of the Canadian one (Nickerson & Murray 1993).

b: Identical PIRTs of Blakemore (Bm) within a column indicates that the relative genotypes were examined simultaneously throughout the research.

c: Not tested

d: Trace of infection present, though with an average PIRT of less than 0.5%.

Table 2. *T*-values for the significance of differences between the PIRT of 14 strawberry genotypes and the respective PIRT of Blakemore for nine USA (A1-A4, A6-A10) and three Canadian isolates (NS2-NS4) of *P. fragariae*[a].

Genotype	Isolate											
	A1	A2	A3	A4	A6	A7	A8	A9	A10	NS2	NS3	NS4
Md683	-6.1	-12.2	-8.7	-8.7	-6.3	-1.0	1.9	-8.8	-5.5	-5.3	-1.7	0.0
Aberdeen-NCSU	-8.2	-7.4	-5.9	-8.5	-8.1	-1.6	-1.4	-8.3	-4.7	-6.1		-0.3
Aberdeen-SCRI	-8.7	-7.4	-0.2	-9.7	-7.8	-10.8	-5.8	-10.0	-5.5	-1.4	-4.9	-1.2
Sparkle	-1.0	-7.4	0.2	-9.7	-1.9	-8.5	0.8	-9.8	-5.2	1.9	-7.2	-1.0
Del Norte	-7.0	-0.3	-6.4	-0.5	-1.3	-8.5	-5.0	-4.5	-1.1	-1.1	-7.2	-7.3
Stelemaster	[b]				-2.6	-5.5	1.5			-9.8	-4.6	-0.4
Yaquina A	-4.8		-4.6	-9.0	-3.9	-6.9		-5.5	-5.3		-6.7	
Perle	-8.7	-11.7	-9.0	-11.4	-8.1	-0.1	-5.5	-6.7	-5.5	-7.3	-2.0	-0.1
Climax	-1.1	-6.9	-1.3	-9.3	-0.8	-11.0	2.9	-8.9	-4.4	0.7	-6.9	0.6
Redgauntlet	0.3	-6.9	0.2	-9.7	-1.3	-11.4	0.2	-10.0	-3.8	0.4	-5.7	2.8
Siletz	-6.7	-6.8	-6.9	-9.7	-7.5	-8.4	0.8	-10.2	-5.0	-7.0		0.0
Auch.6					-3.2	2.0	-1.5			-10.9		-2.2
Yaquina B	-7.0	-7.4	-7.3	-9.7	-8.1	-10.7	-6.4	-9.7	-4.9	-6.4	-4.8	-7.6
S.Sengana	-0.7	0.4	0.3	-2.2	-1.6	-0.1		0.5		0.3	0.8	-0.2

a: Critical *t*-values for the 95%, 99% and 99.5% confidence level are respectively -1.6, -2.3 and -2.6 (769 degrees of freedom).

b: not tested

Table 3. The resistance (-) or susceptibility (+) of 15 host genotypes for 12 single isolates of *Phytophthora fragariae*.

Genotypes	Differential series[a]	USA Isolates									Canadian Isolates		
		A1	A2	A3	A4	A6	A7	A8	A9	A10	NS2	NS3	NS4
Blakemore	U C	+	+	+	+	+	+	+	+	+	+	+	+
Md683	U C	-	Ⓞ[b]	-	-	-	+	⊕	-	-	-	+	+
Aberdeen-NCSU		-	-	-	-	-	+	+	-	-	-		+
Aberdeen-SCRI	U	-	-	+	-	Ⓞ	-	Ⓞ	-	-	+	-	+
Sparkle	C	+	-	+	-	+	-	+	-	-	+	-	⊕
Del Norte	U C	-	+	-	+	+	-	-	-	+	+	-	-
Stelemaster	U C	[c]				-	-	+			-	-	+
Yaquina A	U C	-		-	-	-	-		Ⓞ	Ⓞ		-	
Perle	UK	Ⓞ	-	Ⓞ	-	Ⓞ	⊕	Ⓞ	-	-	-	+	+
Climax	UK	+	-	+	-	+	-	+	-	-	+	-	+
Redgauntlet	UK	+	-	+	-	+	-	+	-	-	+	-	+
Siletz	UK	-	-	-	-	Ⓞ	-	+	-	-	-		+
Auch.6						-	+	+			-		+
Yaquina B		-	-	-	-	-	-	-	-	-	-	-	-
Senga Sengana		+	+	+	+	+	+		+		+	+	+

a: Differential series in which the genotypes are used (U: USA, C: Canadian, UK: United Kingdom)

b: Encircled classifications differ from the USA (Converse 1970) or Canadian series (Nickerson & Murray 1993) or from previous reports on UK differentials (Converse & Scott 1962; Milholland et al. 1989).

c: not tested.

Md683-A2, Aberdeen SCRI-A6, Yaquina A-A9, and Yaquina A-A10. These four combinations showed some traces of infection (Table 1). They were classified as incompatible due to the recognition of incomplete, moderate resistance; the average level of disease of a series of plants was assessed and compared with that of the reference cultivar Blakemore. Consequently, a genotype may still be classified as resistant although a single root of an occasional plant may be severely infected. In contrast, these combinations were previously (Converse & Scott 1962; Converse 1967) classified as compatible, since the infection of the severest infected root of the most severely infected plant extended beyond the root tip. Of two of these four combinations (Md683-A2, Yaquina A-A10) also the average level of disease was reported (Converse & Scott 1962; Converse 1970), as was that of Blakemore-A2 and Blakemore-A10. When these earlier data were evaluated by the present approach, results were identical to those of Table 1 (Van de Weg et al. 1996). A similar evaluation of the other two combinations was not feasible as average disease ratings have not been published.

Md683-A8. When developing the USA differential series, Converse (1967) tested A8 on Auch.6 and credited the result to Md683. The data give support to Converse's assumption on the identity of the resistance of these genetically related selections (Table 4). Consequently, this transfer of Converse does not offer a satisfactory explanation for the difference in classification. However, the consistency of both genotypes with respect to their susceptibility to A8 is in favour of the present result.

Aberdeen-A8. As above, Converse (1967) tested A8 on Sparkle and credited the observed virulence to Aberdeen, presuming them to have the same resistance. Since, this transfer has been either neglected or accepted as justified (Converse 1970; Scott et al. 1984; Nickerson & Maas 1991; Nickerson & Murray 1993). However, one of the two earliest reports on the existence of races of *P. fragariae* already showed their resistance to differ (Scott et al. 1950), as did the present data.

Sparkle-NS4. NS4 was used in a number of experiments during the last 4 years and results show it to be a problematic isolate. Various experiments failed due to too low levels of infection, even in Blakemore. Even when the general level of disease was reasonable, NS4 frequently gave variable results among runner plants of the same genotype, some being severely infected and some remaining symptomless. This variable behaviour might explain why Nickerson & Murray (1993) concluded that cultivar Sparkle was resistant. The validity of the present classification is supported by its consistency with the susceptibility of cultivars Climax and Redgauntlet, genotypes that had identical interactions as those of cultivar Sparkle with all other isolates, and by its consistency with

the susceptibility of Aberdeen (see later).

Siletz-A6. The incompatibility of this combination is also due to the recognition of incomplete resistance. Milholland et al. (1989) used a disease severity index and classified genotypes as susceptible if this index was higher than 1. Siletz was classified as susceptible because it had an index of 2. However, this index differed so vastly from the one of the highly susceptible genotype Tennessee Beauty (index of 65) that the experimental error as the sole cause is highly unlikely. It is therefore that Siletz is resistant to A6.

Perle-A1, Perle-A3, Perle-A6, Perle-A7, and Perle-A8. We could not reproduce the classification by Milholland et al. (1989) of these five combinations. They found Perle to have the same resistance pattern as cultivars Sparkle, Climax, and Redgauntlet, although Perle's resistance had been convincingly discerned in earlier work (Hickman & English 1951; Converse & Scott 1962; Hickman 1962) as well as in recent research (Scheewe 1994) as in the present data (Table 1). Apparently, Milholland et al. used a genotype that was not Perle.
The classifications of Perle-A3 and Perle-A6 differed from those of Converse & Scott (1962) due a different interpretation of disease ratings, as discussed above (section on Md683-A2).

DISCUSSION

Resistance of strawberry genotypes. The identical resistance pattern of the USA differential Sparkle and the British differentials Climax and Redgauntlet is in agreement with earlier reports (Converse & Scott 1962; Milholland et al. 1989). In view of their ancestry (Table 4) the resistance of these genotypes probably originated from Aberdeen. The observation that all isolates virulent to Aberdeen (-SCRI) are also virulent to Sparkle (A3, NS2, NS4), is in agreement with this assumption. However, as part of the isolates virulent on Sparkle are avirulent on Aberdeen (A1, A6, A8), Aberdeen should possess at least one resistance factor in addition to that of Sparkle. Consequently, Aberdeen possesses at least two resistance factors, of which one was passed on to cultivars Sparkle, Climax, and Redgauntlet.
Yaquina-A and Yaquina-B were both resistant to all tested North American isolates, and seem therefore interesting sources of resistance for a breeding programme.

Table 4. Pedigrees of various genotypes. Parental genotypes which are known to possess resistance to *Phytophthora fragariae* var. *fragariae* are printed in bold.

Genotype	Mother	Father	Reference
Auch.6	**No.52** (believed to be Frith)	**No.52**	Reid, 1952
Md683	**BK-46** (= **No.52** x **No.52**)	Fairfax	Scott et al., 1984
Sparkle	Fairfax	**Aberdeen**	Scott et al., 1984
Climax	TD8 (= **No.52** x **No.52**)	**Aberdeen**	Reid, 1952
Redgauntlet	N.J.1051	**Climax**	Scott et al., 1984

Composition of differential series. The USA differential series consists of Blakemore, Md683, Aberdeen, Del Norte, Yaquina A, and Stelemaster. The Canadian set is similar to the USA one except that Aberdeen is replaced by Sparkle. Our results show that these latter genotypes differ in their resistance. Moreover, Sparkle, like Climax and Redgauntlet, is needed to distinguish A1 from A9, and to distinguish A6 from A2, A4, and A10 (Table 3). Aberdeen is needed to differentiate between A1 and A3, between A6 and NS2, and between A8 and NS4 (Table 3); indeed, these isolates could not be differentiated in research in which Aberdeen was absent (Milholland et al. 1989; Nickerson & Murray, 1993). Apparently, Sparkle and Aberdeen should both be included in the USA and Canadian differential series, by which these series become identical.

Perle was not essential for the differentiation of the present isolates. However, it improved the description of the virulence of A7 and NS3. These isolates are virulent on Blakemore, Md683, and Perle. As the latter two genotypes differ in their resistance (Table 3), A7 and NS3 should possess at least two virulences, the presence of one of which is established by Md683, the other by Perle.

Genetics of resistance and avirulence. Van de Weg (1989a) postulated that strawberry and *P. fragariae* var. *fragariae* interact according to the gene-for-gene (GFG) concept of Flor (1956). To date, the design of a reliable GFG-model had been hampered due to variable results among investigators on the resistance of strawberry genotypes and the virulence of fungal isolates (Van de Weg 1989a, 1989b). The present research provided a series of highly reproducible classifications, which should allow the design of a formal GFG-model. This will be presented in a next communication.

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V. A GENE-FOR-GENE MODEL TO EXPLAIN INTERACTIONS BETWEEN CULTIVARS OF STRAWBERRY AND RACES OF *PHYTOPHTHORA FRAGARIAE* VAR. *FRAGARIAE*

ABSTRACT

A gene-for-gene model is postulated to explain the observed interactions between cultivars of strawberry and races of *Phytophthora fragariae*. Five interacting resistance (R1-R5) and avirulence (Avr1-Avr5) factors explain all the available data involving 15 host genotypes, including the USA and Canadian differential series, and 12 pathogen isolates from North America. Interactions between pathogen isolates and UK and German differentials are also explained by the proposed model. The model makes it possible to develop a universally applicable differential series, to present a systematic, unequivocal nomenclature of races, and to increase the efficiency of breeding programs.

INTRODUCTION

Phytophthora fragariae (Hickman 1940) var. *fragariae* (Wilcox et al. 1993) is the causal agent of red stele (red core) root rot in strawberry (*Fragaria* spp.). It is widely assumed that resistance to this soil-borne fungus is inherited polygenically (Stembridge & Scott 1959; Scott et al. 1984). However, the race specificity (e.g. Montgomerie 1967; Converse 1970; Kennedy & Duncan 1993; Nickerson & Murray 1993; Van de Weg et al. 1997a) and the observed Mendelian segregation of resistance (Van de Weg et al. 1989) suggest that genotypes of strawberry and races of *P. fragariae* exhibit a gene-for-gene (GFG) relationship as first described by Flor (1956) for flax and flax rust (*Melampsora lini*). The reports published on host-pathogen interaction data appeared at first too inconsistent (Montgomerie 1967; Van de Weg 1989b; Milholland 1994; Van de Weg et al. 1997a) to derive GFG patterns. However, a number of the inconsistencies have been determined to be due differences in the evaluation of incompletely expressed resistance, to interchanges of cultivars which were incorrectly presumed to have the same resistance, and to the existence of different genotypes under the same cultivar name (Van de Weg et al. 1996, 1997a). In the present article data from which the inconsistencies were removed (Van de Weg et al. 1997a), have been used to derive a GFG model which satisfactorily explains the available observations.

MATERIAL AND METHODS

Data. To the data of Van de Weg et al. (1997a), classifications according to Converse (1970) and Nickerson & Murray (1993) were added (Table 1) and analyzed using methods described by Person (1959). The ability of the resulting GFG model to describe interactions of other cultivar-race combinations was tested on published classifications and on classifications deduced from published disease assessments (see Tables 2 and 3). Host genotypes were classified following Van de Weg et al. (1996), being resistant if they were considerably less diseased than one of the universally susceptible cultivars: Tennessee Beauty and Senga Sengana, or if they scored three or less on the disease severity scale of Kennedy & Duncan (1993).

Resistance and virulence. The GFG hypothesis presumes that for each resistance factor in the host, there is a corresponding avirulence factor in the pathogen (Newton & Andrivon 1995). The product of the resistance allele is postulated to recognize the product of the corresponding avirulence allele, which initiates the resistance response (Keen 1990). In contrast, the product of the virulence allele, if it occurs (De Wit 1992), will not be recognized by the product of the resistance allele, resulting in virulence of the pathogen and susceptibility of the host (Newton & Andrivon 1995). Thus, resistance (incompatibility of host and pathogen genotypes) occurs if a cultivar carries at least one resistance allele for which the pathogen carries the corresponding avirulence allele. Susceptibility (compatibility) requires either the absence of R-alleles or the avoidance of each R-allele in the host by the absence of the matching avirulence alleles in the pathogen.

Denotation. GFG-analysis of interactions between host cultivars and pathogen isolates leads, strictly spoken, to the identification of factors for resistance and avirulence, and not of genes (Person 1959). Therefor it is proposed to delay the use of the latter term until the individual identity of a factor has been substantiated by inheritance studies.
Host genotypes are denoted by their resistance factors, regardless of the number of alleles present. In this way problems arising from variations in ploidy level among *Fragaria* species and the indistinctness of their genome formula are circumvented (Bringhurst 1990; Galletta & Maas 1990). Similarly, pathogen genotypes are denoted by their avirulence factors, which circumvents the indistinctness of the ploidy level of *Phytophthora* species (Brasier 1992).

RESULTS

The resistant and susceptible reactions listed in Table 1 can be explained by a GFG relationship involving five interacting pairs of resistance (R1-R5) and avirulence (Avr1-Avr5) factors. In this model, Blakemore and Senga Sengana have no resistance, while Md683 possesses just R1 (Table 1). Following the GFG concept, all isolates to which Md683 is resistant carry Avr1, while this factor is absent in all isolates to which Md683 is susceptible. Besides Md683, other single-factor genotypes are Climax, Redgauntlet, and Sparkle (all carrying R2), Del Norte (R4), and Yaquina A and Yaquina B (R5). Host genotypes with two factors are Siletz (R1.2), Stelemaster (R1.2), Perle de Prague (R1.3), and Aberdeen (R2.3) (Table 1). Among the host genotypes in Table 1 none contained R3 alone. This prevented the full assessment of isolates A2, A4, A9, and A10 (see footnotes to Table 1).

Sparkle (R2) and Stelemaster (R1.2) are both resistant to A7, as they carry R2 for which A7 has the matching avirulence factor (Avr2). They are both susceptible to A8 (Avr3.4.5), as this isolate lacks Avr1 and Avr2.

Table 1. Resistant (-) and susceptible (+) reactions of twelve strawberry genotypes to nine USA (A) and three Canadian (NS) isolates of *Phytophthora fragariae* var. *fragariae* together with their postulated resistance and avirulence factors. Data are according to Van de Weg et al. (1996, 1997a) with additional data (encircled) from Converse (1970) and Nickerson & Murray (1993).

Host genotypes		Isolates and their proposed avirulence factors								
Name	Proposed resistance factors	A9	A1	A2[c]	A8	A7[d]	A3	A6	NS4	NS2
		1	1	1	.	.	1	1	.	1
		2	.	2	.	2	.	.	.	.
		3?[e]	3	3?	3	.	.	3	.	.
		4	4	.	4	4	4	.	4	.
		5	5	5	5	5	5	5	5	5
Blakemore[a]	0	+	+	+	+	+	+	+	+	+
Md683	1	-	-	-	+	+	-	-	+	-
Sparkle[b]	2	-	+	-	+	-	+	+	+	+
Del Norte	4	-	-	+	-	-	-	+	-	+
Yaquina A	5	-	-	Θ	Θ	-	-	-	Θ	Θ
Yaquina B	5	-	-	-	-	-	-	-	-	-
Siletz	1.2	-	-	-	+	-	-	-	+	-
Stelemaster	1.2	Θ	Θ	Θ	+	-	Θ	-	+	-
Perle de Prague	1. 3	-	-	-	-	+	-	-	+	-
Aberdeen	2.3	-	-	-	-	-	+	-	+	+

a, b, c, d: Genotypes or isolates having identical interactions; a: Senga Sengana; b: Climax and Redgauntlet; c: A4 and A10; d: NS3.

e: Available data allow no conclusion on the presence of the avirulence gene due to the absence of an R3-differential.

The resistances and virulences of UK differentials and isolates can also be explained by the proposed GFG model (Table 2). The same is true for isolates from Germany and the USA, and for various North American strawberry cultivars, except for the reactions of Cardinal, Gilbert, Allstar, Midway, and Crimson King (Table 3). The genotypes 52AC18 (Table 2), MicMac, Blomidon, and Kent (Table 3) are putative R3 differentials. If this holds true, A2 and A4 should possess Avr3 and A9 should not (Table 3).

Table 2. Genotypes of UK strawberry differentials and UK isolates. The incompatibilities of genotype-isolate combinations are deduced from Kennedy & Duncan (1988, 1993) and Kennedy et al. (1986).

Host genotypes	Proposed resistance factors	Isolates and their putative avirulence factors[a]								
		B1 168[b]	B2 172	B4 499	B9 372	B3 169	B11A 171	B10 173	B11B 293	B11D1 452
		1	.	1	1	1	.	.	1	.
		2	2	.	.	2	.	.	.	.
		3	3	3	3	.	3	3	.	.
		4?	4?	4?	4?	4?	4?	4?	4?	4?
		5	5	5	5	5	5	5	5	5
Fragaria vesca	0	+	+	+	+	+	+	+	+	+
Md683	1[c]	-	+	-	-	-	+		-	+
Climax	2[c]	-	-	+	+	-	+	+	+	+
52AC18	3	-	-	-	-	+	-	-	+	+
Yaquina B	5[c]	-	-	-	-	-	-		-	-
Stelemaster	1.2[c]	-	-	-	-	-	+	+	-	+
Aberdeen	2.3[c]	-	-	-	-	-	-	-	+	+
Cambridge Vigour	2.3				-	-	-		+	
Saladin	2.3	-	-		-	-	-		+	+
Perle de Prague	1. 3[c]	-	-	-	-	-	-	-	-	+
Hood	1.2.3	-	-	-	-	-	-	-	-	+
Linn	1. 3 / 2.3 / 1.2.3[d]				-	-	-			+
Tantallon	1. 3 / 2.3 / 1.2.3[d]	-	-		-	-	-			+

a: Although the isolates of this table were also tested on Del Norte (see Table 1), the reported disease ratings were considered insufficiently conclusive. Consequently, no conclusions could be drawn about the presence or absence of Avr4.

b: Culture number of isolate

c: Genotypes of these genotypes are according to Table 1 and are the basis for the assignment of genotypes for the fungal isolates and the other strawberry genotypes.

d: No final conclusion can be drawn on these genotypes as their resistance to isolates B10 and B11B is not known.

Table 3. Genotypes of strawberry cultivars and of isolates from North Carolina (USA) and Germany. The interactions of genotype-isolate combinations are according to Maas et al. (1988, 1989) and Table 1 or deduced from Milholland et al. (1989), Law and Milholland (1992), and Scheewe (1994).

Host genotype	Proposed resistance factors	Isolates, their origin and putative avirulence factors												
		USA differential key							North *Carolina*		Maine	Germany		
		A9	A2	A8	A7	A3	A6	A5	NC1	NC2[4]	Me4K[5]	I1/2	47/1	45/11
		1	1	.	.	1	1	.	.	1	.	1	1	1?
		2	2	.	2	.	.	.	2	2	2	.	.	2
		.	3	3	.	.	3	.	.	3?	.	.	.	3?
		4	.	4	4	4	.	4	4?	4?	4	4?	4?	4?
		5	5	5	5	5	5	5	5	5	5	5	.	5
Blakemore[a]	0[f]	+	+	+	+	+	+	+			+			
Senga Sengana	0[f]	+	+	+	+	+	+					+	+	+
Tennessee Beauty	0	+	+	+	+	+	+	+	+					
Md683	1[f]	-	-	+	+	-	-	+	+	-	+			
Sunrise	1	-	-	+	+	-	-	+	+		+			
Climax	2[f]	-	-	+	-	+	+	+	-	-		+	+	-
Sparkle	2[f]	-	-	+	-	+	+	+	-	-	-			
MicMac[b]	3	+	-	-	+	+	-	+			+			
Del Norte	4[f]	-	+	-	-	-	+	-	?[g]	?	-			
Yaquina A	5[f]	-	-	-	-	-	-	-	-	-				
Yaquina B	5[f]	-	-	-	-	-	-	-	-		-	-	+	-
Aberdeen	2.3[f]	-	-	-	-	+	+	+			-			
Darrow	1.2	-	-	+	?	-	-	+	-					
Lester[c]	1.2	-	-	+	-	-	-	+			-			
Siletz	1.2[f]	-	-	+	-	-	-	-	-					
Stelemaster	1.2[f]	-	-	+	-	-	-	+	-	-	-			
Guardian	1. 3	-	-	-	+[h]	-	-	+	+			-	-	-
Redchief	1. 3	-	-	-	+	-	-	+			+			
Saladin	2.3[f]											+	+	-
Earliglow	1.2/1.2.3	-	-	+/-[i]	-	-	-	+	-		-			
Delite	1.2.3	-	-	-	-	-	-	+	-					
Lateglow	1.2.3	-	-	-	-	-	-	+			-			
Surecrop	1.2.3	-	+/-[i]	+/-[i]	-	-	-	+	-	-				
Tristar	1.2.3	-	-	-	-	-	-	+						
Cardinal	0?	+	+	+	+	+	+	+	⊖[j]					
Gilbert	0?	+	+	+	⊖	+	+	+			+			
Allstar	1?	-	-	+	+	-	-	+	⊖					
Midway	2?	-	-	⊖	?	+	+	+	?					
Crimson King	3?	+	-	⊖	+	+	-	+			+			

a-e: Genotypes or isolates with identical classifications; a: Bounty, Canoga, Honeoye, Jewel, Raritan, and Vesper; b: Blomidon and Kent; c: Annapolis and Cornwallis; d: NC3; e: Me5J and Me7F.

f: Genotypes are according to Tables 1 and 2, they are used for the assignment of genotypes for the other genotypes and isolates.

g: Disease ratings allow no conclusive classification.

h: According to Van de Weg & Henken (unpublished).

i: Inconsistent data among Law & Milholland (1992), Maas et al. (1989), and Milholland et al. (1989)

j: Encircled classifications are inconsistent with the suggested genotypes.

DISCUSSION

A GFG model for the interaction between cultivars of strawberry and races of *P. fragariae* var. *fragariae* is proposed. The model clarifies the genetics of resistance in the host and the genetics of avirulence in the pathogen. It consists of five resistance (R1-R5) and five avirulence (Avr1-Avr5) factors.

Since the GFG relationship was first proposed (Flor 1942; Oort 1944), it has been helpful for the description and interpretation of the genetical relationship in many host-pathogen combinations (Thompson & Burdon 1992), including *Phytophthora* spp.; potato-*P. infestans* (Toxopeus 1956) and soybean-*P. megasperma* var. *sojae* (Ellingboe 1983). Its validity, characteristics, prospects, and limitations have been reviewed frequently (e.g. Flor 1971; Crute 1985; Keen 1990; Heath 1991; De Wit 1992; Newton & Andrivon 1995).

The reliability of the model. The reliability of the present model is supported by its consistency when related cultivars are considered. Climax, Redgauntlet, and Sparkle are likely to have received R2 from their common ancestor Aberdeen (R2.3) (Reid 1952; Scott et al. 1984). The resistance of Stelemaster (R1.2) is also consistent with its ancestry, as it was selected from the cross [Aberdeen (R2.3) x Fairfax (R0)] x Md683 (R1) (Scott et al. 1984). Moreover, the resistance of most North American cultivars created at, or in cooperation with, the Beltsville ARS, MD, USA, strawberry breeding program, traces back to Md683 (R1) and Aberdeen (R2.3). These cultivars were screened for resistance to a composite of the isolates A1, A2, A3, A4, and A6 (Maas et al. 1989), and should therefore have genotypes: R1, R1.2, R1.3, or R1.2.3. This prediction is in agreement with the postulated genotypes of Darrow, Earliglow, Guardian, Lateglow, Lester, Redchief, Stelemaster, and Tristar (Table 3).

The reliability of the model is further supported by recent studies which showed the resistance of Md683 to isolate NS2-25, and the resistance of Climax, Redgauntlet, Sparkle, and Siletz to A7 was monogenically inherited (Van de Weg 1997b; Van de Weg et al. 1997b). The resistance gene of Md683 has been called *Rpf1* and that of the other cultivars *Rpf2*. Caution in the assignment of individual genes to the other resistance factors is proposed until their individual identity has also been substantiated by inheritance studies. Inheritance studies on avirulence factors are impeded by the homothallism (Bain & Demaree 1945) and ploidy level of *P. fragariae* (Brasier 1992). Consequently, the existence of a GFG relationship in the strict sense cannot be proved (Sidhu 1975, Crute 1985).

Sources for resistance in addition to those discussed here have been reported (Reid 1952; Montgomerie 1960; Scott et al. 1962, 1984; Daubeny & Pepin 1965). The testing of these additional sources or new isolates may lead to the identification of more interacting resistance and avirulence factors. It is possible that the number of resistance and avirulence factors in the model will need to be increased if the data recorded for Cardinal, Gilbert, Allstar, Midway, and Crimson King (Table 3) are reproducible.

Nomenclature of races. In other host-pathogen combinations, fungal races are often named according to the virulence factors they carry (De Wit 1992). This system is informative, simple and easily applied provided the number of resistance factors is not too large, as in the case described in this paper. Since virulence is perceived to be the absence of avirulence it is not strictly correct to use the denotation 'virulence factor' or 'virulence gene'; even the deletion of an avirulence locus would result in virulence. Consequently, the concept of virulences is used (Parlevliet 1995) and a nomenclature based on this is proposed. Race 1.3 thus carries virulences 1 and 3 overcoming the resistance factors R1 and R3, isolate A7 being a representative of this race (Table 1).
It should be noted that the assignment of a given isolate to a race is never absolute since new virulences may be identified when the isolate is tested on a larger set of differentials.

Composition of an international set of differential cultivars. The most efficient set for the testing of single-spore isolates consists of host genotypes each possessing a single, unique resistance factor and together comprising all known R-factors (Person 1959; Flor 1971). A universally susceptible cultivar should be included to identify isolates lacking any specific avirulence factors (race Avr0). The set could thus consist of genotypes such as Blakemore (R0), Md683 (R1), Sparkle (R2), Del Norte (R4), Yaquina A (R5), and one of the genotypes assumed to carry R3 (Blomidon, Kent, Micmac, 52AC18). As long as an R3 differential has not been definitively identified, Perle de Prague (R1,3) and Aberdeen (R2,3) can be included (Van de Weg et al. 1997a). This makes it possible to detect the presence or absence of Avr3 in races showing at least one of the virulences 1 and 2.

Current differential sets. The GFG model clarifies the similarities, differences and shortcomings of the current, national differential sets. They consist of cultivars which have been selected for their ability to distinguish isolates, without knowledge of the number or identity of the resistance genes they carry. Therefore, it is not surprising that the composition of these sets is not optimal. The USA set consists of: Blakemore, Md683, Aberdeen, Stelemaster, and Yaquina A (Converse 1970); lacking 'single-factor-genotypes' for R2 and R3. The Canadian set is similar to that of the USA except that Sparkle is used instead of Aberdeen (Nickerson & Murray 1993); lacking an R3 differential, A6 (race 2.4)

could not be distinguished from NS2 (race 2.3.4) (Nickerson & Murray 1993). The set proposed by Milholland et al. (1989) lacked a differential for R3 and R5. This set also failed a differential for R2.3 because the relative clone of Aberdeen seemed not to true to type (Van de Weg et al. 1997a). Consequently, A1 (race 2) could not be distinguished from A3 (race 2.3) (Milholland et al. 1989).

In the UK, various differential sets have been used. They consist of different combinations of the genotypes *Fragaria vesca* (R0), Cambridge Favourite, Climax, Redgauntlet, 53Q13, Del Norte, Yaquina B, Siletz, Perle de Prague, Aberdeen, Cambridge Vigour, Saladin, Talisman, 52AC18, Linn, Hood, and Tantallon (Montgomerie 1967; Kennedy et al. 1986; Kennedy & Duncan 1988, 1993). Only the paper of Kennedy & Duncan (1993) contained all of the required differentials.

Breeding for resistance. The GFG model can help breeders to specify their breeding goals for resistance accurately in terms of desired resistance genes, to decide on the host genotypes to hybridize, and the isolates to screen with. For instance, R1, R2, and R3 can be pyramided by crossing Md683 and Aberdeen and by testing the descendants for resistance to races 2.3 (A3), 1.2 (A8), and 1.3 (A7), and for susceptibility for race 1.2.3 (NS4).

Stability of resistance and distribution of races. The GFG model sheds new light on the previously reported 'breakdown' of resistance. Climax was the first European cultivar originating from a specific breeding program for resistance. However, soon after its release it became severely diseased at many locations, which resulted in an assumption of the occurrence of races (Reid 1948, 1952). Climax derived its resistance from Aberdeen, a cultivar which had remained resistant until that time. This rapid 'breakdown' of resistance may have occurred because Climax carries only one of the two resistance factors present in Aberdeen and because the matching virulence was already widely present prior to the introduction of Climax. To date, virulent races have been found to match each of the presently identified resistance factors. Since not all races yet occur in all growing areas, certain combinations of resistance genes will still be effective in red stele-infested areas.

Resistance factor R5 seems to be effective in many growing areas since Yaquina B was resistant to all 300 European isolates examined by Kennedy & Duncan (1993), and to a total of 48 North American isolates reported on by Converse (1970), Milholland et al. (1989), and Nickerson & Murray (1993). However, this factor seems to be less effective in Germany and The Netherlands as it was not effective against one out of three German isolates (Table 3) and one out of two Dutch isolates tested by Van de Weg (unpublished).

The resistance conferred by R1.2.3 seems also to be highly effective in many areas. Compatible isolates are found only occasionally. The first was isolated in 1964 in the Eastern United States as isolate A5 (Converse et al. 1966). Since then it has been isolated once more in the USA (Converse et al. 1966), Canada (isolate NS4) (Nickerson & Maas 1991; Nickerson & Murray 1993) and on three occasions in Europe (isolate B11-D) (Kennedy & Duncan 1993). Evidently, this race has never established itself widely during the past 40 years. The low zoospore-producing capacity of these isolates (Maas 1976a; Nickerson & Murray 1993), their low aggressiveness (Maas 1976b; Milholland et al. 1989; Van de Weg et al. 1997a), and instability (Kennedy & Duncan 1988, 1992) might be reasons for this.

The resistance conferred by R1.3 also seems to be effective in many areas. Race 1.3 has had a limited distribution for a long time, while more complex races are extremely rare (see above). This pathotype was not represented among the 300 European isolates tested by Kennedy & Duncan (1993). In the USA, it initially appeared on the west coast only. However, it was recently detected on the east coast of both the USA (Maas et al. 1988; Milholland et al. 1989) and Canada (Nickerson & Murray 1993), overcoming the resistance of some modern cultivars (Maas et al. 1988).

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VI. RESISTANCE TO *PHYTOPHTHORA FRAGARIAE* VAR. FRAGARIAE IN STRAWBERRY: THE *Rpf2* GENE.

ABSTRACT

Phytophthora fragariae var. *fragariae* is the causal agent of red stele (red core) root rot in strawberry (*Fragaria* spp.). The inheritance of resistance to one isolate of this fungus was studied in 12 segregating populations of *F.* x *ananassa* derived from crosses between four resistant cultivars (Climax, Redgauntlet, Siletz, and Sparkle) and three susceptible cultivars (Blakemore, Glasa, and Senga Sengana). The analysis clearly supports the hypothesis of a single segregating dominant resistance gene. It is proposed that this gene be designated *Rpf2*.

INTRODUCTION

There is increasing evidence that cultivars of strawberry and races of *Phytophthora fragariae* var. *fragariae* (Wilcox et al. 1993) interact in a gene-for-gene (GFG) manner as described by Flor (1956) for flax and flax rust (*Melampsora lini*). A GFG model has been proposed for five pairs of interacting resistance factors of the host and avirulence factors of the pathogen based on the analysis of a large number of genotype-race combinations (Van de Weg 1989, 1997). Although within GFG systems, resistance and avirulence factors are generally based on single genes (De Wit 1992; Thompson & Burdon 1992), their genetic basis has to be formally assessed by genetic analysis.

The cultivated strawberry *Fragaria* x *ananassa* is an allo-octoploid (2n=8x=56) originating from a natural hybridisation event between the allo-octoploid species *F. chiloensis* and *F. virginiana*. While the genome structure of these species is still unresolved (see Galletta & Maas 1990), various structures have been suggested: AABBBBCC (Fedorova 1946) and AAA'A'BBBB (Senanayake & Bringhurst 1967). These allow di- and tetrasomic segregation to occur simultaneously within a single progeny. In the case of tetrasomy, easily interpretable segregation ratios may occur if the trait of interest is governed by a qualitative, dominant gene. Race specific resistance has frequently been shown to be such a trait in other host-pathogen systems (Thompson & Burdon 1992).
In the investigation presented here inheritance of the race-specific resistance of the strawberry cultivars Climax, Redgauntlet, Sparkle, and Siletz to the fungal isolate A7 is examined. On the basis of the GFG model (Van de Weg 1997) these cultivars were expected to possess a single resistance factor (2) for this isolate.

MATERIAL AND METHODS

Plant material. Twelve strawberry (*Fragaria* x *ananassa*) progenies segregating for resistance factor 2 (Van de Weg 1997) were created by crossing four resistant genotypes (Climax, Redgauntlet, Sparkle, and Siletz) with three universally susceptible genotypes (Blakemore, Glasa, and Senga Sengana) (Table 1). In addition, 4 highly susceptible progenies were created; 3 by crossing among the susceptible genotypes mentioned above, and 1 by crossing Senga Sengana and Md683. Md683 carries resistance factor 1 which is not effective against the isolate used in the present study (Van de Weg 1997a). Climax, Redgauntlet, and Sparkle are interrelated and received their resistance from their common ancestor Aberdeen. They arose from the crosses TD8 x Aberdeen, N.J. 1051 x Climax, and Fairfax x Aberdeen, respectively (Reid 1952, Scott et al. 1984).

All pollinations were made in the glasshouse on emasculated flowers, which were enveloped with a parchment bag from emasculation on until the receptacles were clearly swelling. Resistant parents were used as the female, except in the crosses Glasa x Climax and Senga Sengana x Climax. Seeds were stratified for 8 weeks at 2 °C, and sown in an autoclaved mixture of sand and peat based compost. Seedlings were raised under standard conditions.

The 12 progenies segregating for resistance will be referred to as the RxS progenies, regardless of the direction of the cross. The 4 highly susceptible progenies will be referred to as SxS progenies.

Disease test. Seedlings with two to five true leaves were lifted and inoculated with isolate A7 [race 1.3 (Van de Weg 1997a)]. Experimental procedures and conditions were according to Van de Weg et al. (1996), with two modifications: inoculated seedlings were potted in smaller plastic pots (70 ml), and the period in which they stood in a shallow layer of water (2 to 7 mm) after inoculation was extended from 3 days to 6 weeks. The layer was replenished by watering on the soil of the plants.

Disease assessment. Six weeks after inoculation, seedlings were lifted and the soil was rinsed off the roots. Adventitious roots with any external or internal discoloration were examined microscopically to detect oospores (Van de Weg et al. 1996). Seedlings with more than three oospores were classified as susceptible, all others as healthy, being resistant or escapes from infection (see discussion).

Experimental design. The 3 RxS progenies from a single resistant parent (Table 1) were tested simultaneously in each of two replicative experiments. Since the research included four resistant parents (Table 1), a total of eight experiments was performed. Each of these

experiments also included 3 SxS progenies (Table 2). Within an experiment, each of the 3 RxS and 3 SxS progenies were tested by 72 seedlings.

Statistical analysis. In resistance tests involving *P. fragariae*, some susceptible plants often remain healthy due to their escaping from infection (Draper et al. 1970; Maas 1970; Scheewe 1994; Van de Weg et al. 1996). Consequently, the proportion of healthy (*H*) seedlings depends on the proportion of genetically resistant seedlings (*GR*) and the proportion of susceptible seedlings which escaped from infection (*E*) according to the formula: $H = GR + (1 - GR) * E$, which can also be written as $GR = (H - E) / (1 - E)$. As *H* can be assessed in the RxS progenies and as *E* can be deduced from the SxS progenies, *GR* can be calculated.

Effects of the parentage on percentages of healthy and resistant seedlings were analysed by means of generalised linear models using a binomial distribution and a logit link function and tested at the 95% confidence level. The statistical package GENSTAT (Genstat 5 committee 1987) was used for analyses.

RESULTS

Percentages of healthy seedlings of the RxS and SxS progenies are presented in Table 1. In the analysis of the SxS progenies, no significant parental effects were found, indicating that all SxS progenies were equally susceptible, which enabled their data to be pooled within experiments. Experimental effects were highly significant ($P = 0{,}006$), with the percentages of healthy seedlings (escapes) ranging from 2% in experiment III up to 24% in experiment I (Table 1). In the analysis of the RxS progenies, percentages of healthy seedlings were around 50%, with the exception of those in experiment I, which were around 60%. The latter experiment also showed the highest percentage of escapes in the SxS progenies (Table 1).

Estimations for the percentages of resistant seedlings were obtained by adjusting the percentages of healthy RxS seedlings for escapes (using the formula given in 'statistical analysis'). These estimated percentages were around 50% for all of the 12 RxS progenies (Table 2), in their analysis neither the resistant nor the susceptible cultivars showed significantly different parental effects. In addition, differences among the 12 RxS progenies were also not significant, i.e. there was no significant interaction between resistant and susceptible parents. These results are expected for the segregation of a single major resistance allele.

Table 1. Percentages of healthy strawberry seedlings of (A) 12 RxS progenies descended from crosses between four resistant and three susceptible cultivars, and of (B) 4 SxS progenies of which the parents have no effective resistance, in eight tests for resistance (I - VIII) with isolate A7 of *Phytophthora fragariae* var. *fragariae*.

Susceptible cultivar	Resistant cultivar and experiment							
	Climax		Redgauntlet		Sparkle		Siletz	
	I[a]	II	III	IV	V	VI	VII	VIII
A: RxS progenies								
Blakemore	60	46	46	44	46		49	54
Glasa 61	54	56	54	54	54	52	44	
S.Sengana	61		53	49	56	49	50	58
Average	60	50	51	49	53	52	52	51
B: SxS progenies								
Blakemore x S.Sengana	27		3	7	8	6	7	7
Blakemore x Glasa	17	10	3	12	17	17		
S.Sengana x Glasa	27	6	1	6	12	17	7	14
Md683 x S.Sengana							4	6
Average	24	8	2	8	12	12	6	9

a: The SxS progenies of experiments I and II, were tested simultaneously with the RxS progenies of Climax, those of III and IV, V and VI, and VII and VIII were tested simultaneously with the progenies of Redgauntlet, Sparkle, and Siletz, respectively.

Table 2. Estimated percentages of resistant seedlings of 12 strawberry progenies when tested against isolate A7 of *Phytophthora fragariae* var. *fragariae*.

Susceptible parent	Resistant parent				Mean
	Climax	Redgauntlet	Sparkle	Siletz	
Blakemore	44	41	39[a]	48	**43**
Glasa	49	52	48	44	**48**
S.Sengana	48[b]	47	46	51	**48**
Average	**48**	**47**	**46**	**48**	**47**

a, b: tested by 39 and 66 seedlings respectively instead of 144.

DISCUSSION

The 1:1 segregation ratio of the progenies provides Mendelian evidence for the presence of a single-copy of a resistance gene in Climax, Redgauntlet, Sparkle, and Siletz. Since the former 3 cultivars are interrelated and since no differences in resistance among them have been found when tested to a series of 12 mutually unrelated isolates (Van de Weg et al. 1997), it is concluded that they possess the same resistance gene. The respective allele must be dominant since Climax upon selfing gives rise to both resistant and susceptible seedlings (Reid 1952), whereas recessiveness should have led to resistant plants only. It is proposed that this gene be designated *Rpf2* (see below). The resistance of Siletz should also be due to *Rpf2* according to the GFG model (Van de Weg 1997).

Theoretically, 1:1 segregation ratios can also occur with certain combinations of complementary genes of a di- or oligogenic trait. For instance, two complementary genes should yield a 1:1 ratio if they exhibit independent and disomic inheritance, with one of them being homozygous and the other heterozygous for the resistance allele, and with a nulliplex susceptible genotype for both genes. It is highly unlikely that such specific conditions occurred by chance in all 4 resistant genotypes. Whereas this probability is already small for 4 randomly chosen resistant genotypes, it is minute for cultivars of subsequent generations originating from RxS crosses, like Climax and Redgauntlet (N.J. 1051 x Climax). In this progeny one of the alleles of the homozygous gene should have come from the susceptible parent (which is N.J. 1051 for Redgauntlet). Due to the low probability of this and on the bais of other alternative genetic models, *Rpf2* should be single-gene based.

The data do not distinguish di- and tetraploid inheritance since the observed 1:1 ratio always occurs in simplex x nulliplex crosses, irrespective of the ploidy level. Conclusions regarding the ploidy level at which *Rpf2* segregates can be drawn only by examination of duplex times nulliplex crosses, or of the cosegregation of linked alleles (repulsion phase).

Percentages of resistant seedlings. Estimates of the percentages of resistant seedlings are affected by three types of error: the frequency at which susceptible seedlings escape from infection, the frequency at which resistant seedlings show some infection, and the frequency of inaccurate disease assessments.

Escapes. Frequencies of escapes were derived from the percentages of healthy seedlings of SxS crosses (Table 1). That such seedlings are really escapes was shown by a test in which all but 1 of the healthy SxS seedlings of experiment III (Table 2) became severely diseased after a second inoculation. As the 4 SxS progenies were equally susceptible to

A7, inclusion of just a single SxS progeny as reference for susceptibility should suffice in future.

The frequency of escapes is affected by inoculum density (Maas 1970). In a preliminary experiment it increased from 10% to 50% by decreasing the inoculum density by 90% (Table 3). However, the estimated percentages of resistant seedlings were similar with both densities (Table 3), showing once more that adjustment for escapes of the percentages of healthy RxS seedlings leads to consistent segregation ratios.

Table 3. Percentages healthy and resistant strawberry seedlings of two progenies, one segregating for resistance (RxS), and one entirely susceptible (SxS) to isolate A7 of *Phytophthora fragariae* var. *fragariae*, at two inoculum densities[a].

Progeny	% Healthy		% Resistant	
	Inoculum density		Inoculum density	
	Standard	Diluted[b]	Standard	Diluted
Redgauntlet x Glasa (RxS)	51	75	46	50
Senga Sengana x Glasa (SxS)	10	50		

a: All treatments were tested simultaneously. Experimental procedures and conditions were as for the other experiments. Each progeny-density combination was tested using 96 seedlings.

b: standard inoculum density diluted with distilled water in a 1:9 ratio by volume.

Infection of resistant seedlings. In this investigation the distinction between susceptible and healthy seedlings was based on the presence or absence of a few oospores. This classification may lead to a slight underestimation of the actual percentage of resistant seedlings since *Rpf2* does not always fully exclude infection. In previous research (Van de Weg et al. 1997) 98.6% of the adventitious roots of the 4 resistant cultivars remained healthy, and 1.4% (7 out of 491) was slightly infected by A7. Here, seedlings generally had three or four adventitious roots at the time of observation. Assuming that here too each root had a 98.6% chance of remaining healthy, the chance that seedlings with three adventitious roots remained healthy should be $0.986^3 = 0.958$, which is 95.8%. In the case of 4 adventitious roots this would be $0.986^4 = 0.946$, which is 94.6%. Consequently, between 4.2% and 5.4% of the resistant seedlings will be misclassified as susceptible. This would reduce the percentages of resistant seedlings to be observed, which should be 47.9% (= 50% x 0.958) and 47.3% (= 50% x 0.946) in the case of a 1:1 segregation. These expected percentages are very similar to the observed ones (Table 1), confirming the degree to which resistant seedlings are correctly identified and the monogenecity of *Rpf2*. Apparently, the present approach of disease assessment suffices for screening populations segregating for *Rpf2*.

Disease assessment. Disease assessment included microscopic establishment of the presence of oospores, their appearance being the most dependable proof for the presence

of the fungus (Bain & Demaree 1945). The necessity of microscopic observations for highly accurate disease assessments was supported by the correlation between these microscopic data and additional macroscopic observations based on the degree at which steles were reddened (data not shown). This reddening was assessed on a discrete scale of 2 (severely diseased) to 10 (no symptoms), following Converse & Scott (1962). The correlation fitted for 92.5% of the seedlings; of the deviating 7.5%, 2% had no oospores but showed red steles, 0.5% had oospores but showed no reddening, and 5% could not be rated macroscopically due to indistinct symptoms.

The macroscopic observations confirmed the dichotomy of the RxS progenies, as 46% of their seedlings rated 2, 51% rated 10, and 3% had intermediate ratings (data not shown).

Nomenclature. In strawberry, a convention on the denotation of genes still fails. Here, the symbol *Rpf* follows the gene nomenclature of Yoder (1986) and Søgaard & Von Wettstein-Knowles (1987) for genes in barley. The 'R' refers to a resistance gene of the host, while the 'p' and 'f' are the first letter of the generic (p) and varietal name (f) respectively, of the pathogen to which this gene confers resistance. The italicised symbol indicates a gene or locus, the nonitalicised symbol indicates the relative phenotype. The first letter is capitalised to indicate dominance of the resistance allele. The number refers to the resistance factor of the GFG model (Van de Weg 1997).

Genetics of other traits. Elucidation of the genetics of commercially interesting traits in the cultivated strawberry has for long been assumed hardly feasible due to its octoploidy ($2n=8x=56$). For some traits, however, the genetics has recently been elucidated. Arulsekar et al. (1981) showed that the isozymes phosphoglucoisomerase (PGI) and leucine amino peptidase (LAP) are governed by a single gene. Next, Van de Weg et al. (1989) found the resistance of various North American genotypes to *P.fragariae* var. *fragariae* to be inherited monogenically. Also, day-neutrality and sex (Ahmadi et al. 1990; Ahmadi & Bringhurst 1991), and resistance to *Colletotrichum acutatum* (Denoyes-Rothan personal communication) were found to be monogenic traits. The present report adds the resistance of *Rpf2* to this list. Results like these can be expected for monogenic traits for which one of the crossing parents has a single dominant allele, since this allele is passed on to half the gametes and should thus result in a 1:1 segregation ratio, independent of the ploidy level. Since the cultivated strawberry is an amphipolyploid, and not a true autopolyploid (Bringhurst 1990; Galletta & Maas 1990), such clear segregation ratios are likely to occur relatively frequently. These observed and the theoretically anticipated successes will hopefully encourage genetic research on other traits.

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VII. RESISTANCE TO *PHYTOPHTHORA FRAGARIAE* IN STRAWBERRY: THE *Rpf1* GENE.

ABSTRACT

The genetics of resistance was examined of the strawberry (*Fragaria* x *ananassa*) genotype Md683 to *Phytophthora fragariae* var. *fragariae*, the causal agent of red stele (red core) root rot. A cross between the incompletely resistant genotype Md683 and the susceptible cultivar Senga Sengana yielded an offspring of 30 resistant and 33 susceptible genotypes for the fungal isolate NS2. This segregation does not significantly differ from the 1:1 ratio supporting the hypothesis of a single resistance gene, which is now designated *Rpf1*.

The disease severity threshold used to discriminate between resistant and susceptible F_1 genotypes was based on the distribution of disease ratings of the resistant parent Md683 relative to that of the universally susceptible cultivar Blakemore. When this approach was applied to the literature, the presence of a single gene was recognized in Md683 for resistance to three other fungal isolates (A1, A2, and A3). This gene should be the same *Rpf1* gene according to the gene-for-gene model for the strawberry-*P. fragariae* pathosystem.

INTRODUCTION

Phytophthora fragariae var. *fragariae* (Wilcox et al. 1993) is the causal agent of red stele (red core) root rot in strawberry (*Fragaria spp.*). It has been suggested that races of this fungus and cultivars of strawberry interact in a gene-for-gene (GFG) manner as described by Flor (1956) for flax and flax rust (*Melampsora lini*). A GFG model for five pairs of interacting resistance and avirulence factors satisfactorily explained a series of cultivar-race interaction data (Van de Weg 1997a). Although within GFG systems, resistance and avirulence factors are generally based on single genes (De Wit 1992), their genetic basis has to be formally assessed by genetic analysis. Such research has been performed for resistance factor 2, which was accordingly designated *Rpf2* (Van de Weg 1997b). Here the inheritance is examined of resistance factor 1 of the GFG model present in the strawberry genotype Md683 (Van de Weg 1997a).

MATERIALS AND METHODS

Plant material. The strawberry (*Fragaria* x *ananassa*) genotypes Md683 and Senga Sengana were crossed according to Van de Weg (1997b). From the resulting seed, 63 F_1

genotypes were raised. Md683 supposedly has resistance factor 1, while Senga Sengana is universally susceptible to *P. fragariae* (Van de Weg 1997a).

Disease test. Genotypes were tested against isolate NS2-25, which is avirulent on Md683 (Nickerson & Murray 1993; Van de Weg et al. 1996). Inoculation procedures and experimental conditions were according to Van de Weg et al. (1997b), with one modification: the period in which inoculated plants stood in a shallow layer of water (2-7 mm) was extended from three days to six weeks. The layer was replenished every other day by watering on the soil of the plants. Six weeks after inoculation plants were lifted and the soil was rinsed off the roots. Main roots with any external or internal discoloration were examined microscopically for the presence of oogonia and oospores. The root segment between the most proximal oospore and the root tip was considered to be affected due to infection by *P. fragariae*. The percentage of affected root tissue was estimated and scored on a discrete scale of 0 to 6: score 0, no symptoms; score 1, less than 4% of the total length of the main roots affected; score 2, 4-10%; score 3, 11-25%; score 4, 26-50%; score 5, 51-75%; score 6, 75-100%.

Experimental design. The experiment was performed with two replicates in time. Each replicate consisted of two blocks, each of which included one runner plant of each F_1 genotype, thus giving four runner plants per genotype over the two replicates. In addition to the F_1 genotypes, two reference genotypes were tested, i.e. the resistant genotype Md683 and the universally susceptible genotype Blakemore. Senga Sengana could not be included as susceptible reference due to a shortage of runner plants.

RESULTS

No significant experiment or block effects were found, therefore the disease score for each F_1 genotype was based on the average score of the four runner plants. The distribution of these average scores is presented in Table 1. The Md683 x Senga Sengana progeny can clearly be separated into two groups, one scored similarly to the resistant parent Md683, and the other to the susceptible cultivar Blakemore. The resistant group contained 30 F_1 genotypes, the susceptible group 33. These numbers do not deviate significantly from the 1:1 segregation ratio ($\chi_1^2 = 0.01$, $P > 0.99$), providing Mendelian evidence for the presence of a single locus for resistance in Md683, designated *Rpf1*.

Table 1. Distribution of the average disease severity by *Phytophthora fragariae* of 63 F_1 genotypes from the cross Md683 (resistant) x Senga Sengana (susceptible) and of runners of Md683 and the susceptible cultivar Blakemore.

Average Disease Rating[a]	Number of F_1 genotypes	Number of runners of	
		Md683	Blakemore
0	6 ┐	9	
>0 - 1	13 ├ 30	6	
>1 - 2	11 ┘	2	
>2 - 3			1
>3 - 4	1 ┐		
>4 - 5	3 ├ 33		3
>5 - 6	29 ┘		10

a: see material and methods.

DISCUSSION

Md683 appears to possess a single allele (*Rpf1*) for resistance towards isolate NS2-25 of *P. fragariae* var. *fragariae*. This allele is supposed to be dominant since Md683 upon selfing gave rise to both resistant and susceptible seedlings (Scott et al. 1950), whereas recessiveness should have led to resistant plants only. The *Rpf1* gene confers incomplete resistance (Table 1). Its designation follows that of the earlier identified resistance gene *Rpf2* (Van de Weg 1997b), following the nomenclature by Søgaard & Von Wettstein-Knowles (1987). The data substantiate the proposed GFG model (Van de Weg 1997a), which predicted the presence of a single resistance gene in Md683.

Md683 has been one of the three most important sources of resistance for *P. fragariae* of the USDA strawberry breeding program at Beltsville, MD. Its resistant offsprings have been used as source for resistance in other breeding programs, including that of CPRO-DLO, and that of Agricultural Canada at Kentsville, NS. The presently acquired insight on the monogenecity of its resistance will facilitate its incorporation into new cultivars.

Incompleteness of the *Rpf1* resistance. A considerable part of the runners of Md683 became slightly infected by the avirulent isolate (Table 1). As a consequence, estimates of the percentages of resistant F_1 genotypes are affected by the level of the disease threshold used to distinguish resistance and susceptibility. Here, scores of 2 and lower indicated resistance, those higher than 3 susceptibility. This distinction was deduced from the discontinuity in the distribution of the disease scores of the F_1 progeny, and from the gap between the scores of the resistant and susceptible reference cultivars.

Earlier inheritance studies. The presently observed numbers of resistant and susceptible F_1 genotypes also allow a 3:5 segregation ratio (95% confidence level) because of the low progeny size. This ratio would require alternative genetic explanations, e.g. the presence of two complementary dominant genes in an RxS cross in which the resistant parent has both genes in simplex condition, and in which the susceptible parent possesses just one of these genes, also in simplex condition. However, earlier inheritance studies are in support to the monogenic 1:1 ratio, as will be illustrated. The presence of a single segregating gene could remain concealed due to not-recognition of the incompleteness of the *Rpf1*-resistance.

Stembridge (1961). Stembridge examined the inheritance of the resistance of Md683 to isolate A1, A2, and A3, scoring disease severity on a discrete scale of 1 (severely diseased) to 6 (no symptoms) where scores of 4 to 6 were thought to indicate resistance in all tests. In the tests for A1, the entire SxS progeny scored 1 (Table 2). Consequently, we concluded that not only scores of 4 to 6, but also scores of 2 and 3 indicated resistance. Following this classification, 89 (54.6%) seedlings of the RxS cross Midland x Md683 were resistant and 74 were susceptible. These numbers do not differ from the 1:1 segregation ratio expected for a monogenic trait ($\chi_1^2 = 1.4$, $P > 0.90$).
In the test for A2 almost all SxS seedlings scored 1, due to which score 1 is thought to indicate susceptibility and scores of 2 and up resistance. Consequently, 87 and 82 seedlings are classified as respectively resistant and susceptible, which numbers do also not differ from the 1:1 ratio too ($\chi_1^2 = 0.1$, $P > 0.99$).
In the test for A3, almost all SxS seedlings scored 3 and lower, while runner plants of Md683 scored 4 or higher. Apparently, scores 1 to 3 indicated susceptibility and scores 4 to 6 resistance. Consequently, 85 (44.0%) seedlings of Md683 x Howard 17 should be classified resistant and 108 susceptible. These numbers once again do not differ from the 1:1 ratio ($\chi_1^2 = 2.7$, $P > 0.95$). We conclude that, contrary to Stembridge's (1961) interpretation, all these data are consistent with each other and that they provide further evidence for our hypothesis of a single resistance allele for A1, A2, and A3 in Md683.

Melville et al. (1980). Melville et al. examined segregation ratios for resistance in 25 RxR crosses of a half dialel using a mixture of five isolates (A1, A2, A3, A4, and A6). The parents were inbred and non-inbred strawberry selections which were completely resistant to the mixture, and which had Md683 (*Rpf1*) and Aberdeen (*Rpf2*,R3) in their ancestry. They considered four classes: resistant (R), intermediate (I), susceptible (S),and very susceptible (VS) and analyzed the percentages of class 'R', which varied between 57.5% and 78.3%.

In the light of this thesis it can be understood that the resistance of all the parental selections should have been due to *Rpf1* (chapter 5). Considering the incompleteness of the *Rpf1* resistance, it is likely that part of the *Rpf1*-seedlings rated 'I'. The percentage of

Table 2. Distribution (in numbers) of disease scores following inoculation with three isolates of *Phytophthora fragariae* var. *fragariae* of RxS[a] crosses as well as of some SxS[a] crosses or selections which are used as reference for susceptibility or resistance. Data were derived from Stembridge (1961).

Disease rating[b]	Isolate							
	A1		A2			A3		
	Midland x Md683	Midland selfed	Md683 Howard 17	Midland Selfed	Md683 runners	Md683 Howard 17	Midland selfed	Md683 runners
	SxR	SxS	RxS	SxS	R	RxS	SxS	R
1	74 — 74	176	82 — 82	32		48 ┐	142	
2	10 ┐		20 ┐	1		13 ├ 108	11	
3	49 ├ 89		50 ├ 87	1	4	47 ┘	25	
4	28 ┤		17 ┘		8	53 ┐	3	7
5	2 ┘					32 ┴ 85		5
6								

a: R: resistant cultivar, S: susceptible cultivar

b: score 1: more than 75% of the root system infected, or with reddened steles extending to the crown; score 2: 50%-75%, score 3: 25% - 50%, and score 4: less than 25% of the root system infected; Score 5: infection suspected, but not confirmed by reddened steles above the portions of externally decayed roots; Score 6: no visible symptoms (Stembridge 1961).

Table 3. Percentage of symptomless to moderately diseased seedlings of 25 progenies derived from crosses among inbred (S) and non-inbred (N) strawberry MdUS-selections when tested to mixture of five US-isolates (A1-A4,A6) of *P. fragariae*. Each cross consisted of 750 seedlings. Data were derived from Melville et al. (1980).

Parents[a]	Parents					
	4355 (N)	4426 (N)	4509 (S_1)	4515 (S_1)	4519 (S_2)	4520 (S_2)
4355 (N)	73.3	81.6	70.5	76.2	75.4	79.1
4426 (N)		73.7	82.1 79.6c	79.2 82.5c	80.4	72.0
4509 (S_1)[b]			73.0	81.8 77.9c	73.7	70.8
4515 (S_1)				70.8	80.4 80.5c	77.1
4519 (S_2)					75.9	79.9
4520 (S_2)						76.7
Average % of all progenies of a selection	76.0	79.9	76.6	78.5	78.0	75.9[d]
Overall average						77.6

a: In this table no account is taken for the direction of the cross

b: derived after one (S_1) or two (S_2) generations of selfing of a resistant selection

c: reciprocal cross

d: Though the deviations to the expected 75% were small to very small, they were too large and to consistent to be explained solely by sampling errors. Therefore it is expected that approximately 10% (average) of the genetically susceptible *rpf1*-genotypes escaped from severe infection and scored 'R' or 'I'.

seedlings in the joined classes 'R' and 'I' were all close to 75% (Table 3). We therefore conclude that, contrary to the earlier interpretation, these data are consistent with the presence of a single effective resistance allele in each of the parents.

The finding that all four inbred genotypes are simplex for *Rpf1* is not amazing, since it had a chance of 9% to occur, assuming that *Rpf1* was also present as simplex in the initial S_0 parents.

Test for resistance. The test for resistance clearly recovered the known difference in resistance between Md683 and Blakemore, and it resulted in a clear segregation in the F_1 progeny (Table 1). It approved to be highly reliable and has now mainly replaced an earlier, more accurate but more laborious method (Van de Weg et al. 1996) in the ongoing red stele studies at CPRO-DLO. Its main modification involves the methodology of disease assessment. Currently, the percentage of *affected* root tissue is considered instead of the percentage of invaded tissue. Also, this percentage is now estimated and assessed on an ordinal scale whereas the earlier method assessed the relative percentage precisely. Due to these simplifications, microscopical examinations could be limited to the most proximal part of infected root tissue, whereas in the previous approach the entire length of each discoloured root segment had to be scanned microscopically.

Number of plants. In this test F_1 genotypes were evaluated on the basis of four runner plants. Almost all would have been identically classified if just a single runner plant was used. When runners of a score of 3 and higher are considered as susceptible, 6% of the runners from a resistant genotype would have been misclassified as susceptible, while 2% of the runners from a susceptible genotype would have been classified as resistant because they escaped severe infection (Table 4). These low percentages of misclassification of individual plants are acceptable in initial resistance tests in breeding programs, although they may be too high to establish molecular markers linked to *Rpf1*.

Table 4. Distribution (in percentages) of disease severity by *Phytophthora fragariae* of individual runner plants of resistant and of susceptible F_1 genotypes descending from the cross Md683 x Senga Sengana.

Disease Rating	Resistant F_1 genotypes	Susceptible F_1 genotypes
0	44	1
1	36	1
2	14	
3	6	
4		4
5		18
6		76

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GENERAL DISCUSSION

The establishment of a gene-for-gene relationship between strawberry and *P. fragariae* is a major step in the elucidation of the genetics of resistance to *P. fragariae*, as is the identification of the two highly effective, race specific resistance genes *Rpf1* and *Rpf2*. The proposed GFG-model is the first for a soil-borne fungus, and the second for a soil-borne pathogen (Janssen et al. 1991). Its genes are the first identified resistance genes in strawberry.

Besides a GFG-model, this thesis also proposes methodologies for assessment of the resistance of strawberry genotypes and for estimating the proportion of resistant descendants in inheritance studies. Their most critical elements are inclusion of a susceptible reference cultivar or SxS cross to account for escapes as well as for the occurrence of incomplete resistance. Their value was demonstrated in the construction of the GFG-model (chapters 4 & 5), and the analysis of segregation ratios for resistance in inheritance studies (chapters 6 & 7). The initially proposed highly quantitative but laborious approach of disease assessment (chapter 2) could be replaced by more simple ones (chapters 6 and 7) once the effectiveness of the resistance genes under study was known.

The fungal isolates used in this study were all of North American origin. This material has been so widely examined, that the relative data could serve as a critical touchstone for the validity of new approaches, the development of which was a major goal in this research. These isolates were therefore preferred over European ones, taking for granted that this thesis would itself not directly contribute to the elucidation of the identity of European isolates.

RESISTANCE

Incomplete resistance. The occurrence of incomplete resistance to *P. fragariae* an its potential value for an economic and environmental healthy production of strawberries on *P. fragariae* infested soils has since long been recognized (Waldo 1953; Reid 1956; Gooding 1972; Galletta et al. 1989). In addition, Hickman & English (1951b) and Hickman (1962) recognized the relevance of incompleteness of pathogenicity for race differentiation. The present research built on these early experiences by considering levels of resistance and pathogenicity quantitatively. The validity of this approach is substantiated by the results, which showed that resistance conferred by *Rpf1* and R3 is incomplete, while that conferred by *Rpf2* is close to immunity. The difference in effectiveness among these genes clarifies the general consistency among previous data

where *Rpf2* was involved, and the frequent inconsistencies where *Rpf1* was involved (chapters 4 & 5).

Earlier appreciation in race differentiation. Hickman (1962) showed the relevance of quantitative disease assessments for race differentiation, which approach has since not been continued. In addition to differentiation of races based on absolute pathogenicity towards the cultivar Climax, he further differentiated some isolates on the basis of their degree of pathogenicity towards the cultivar Perle de Prague. These isolates felt into three groups; slightly pathogenic, pathogenic, and highly pathogenic. In the present study this quantitative approach is revalued since Hickman's observations can now be understood by the presence of two race specific resistance genes in Perle de Prague, which genes differed in effectiveness. Isolates which were arrested by the more effective, incomplete resistance gene would have been classified as slightly pathogenic, those avirulent to the less effective gene as pathogenic, and those not retarded at all, because they were avirulent to both resistance genes, as highly pathogenic.

Stability of resistance. Virulent races have already been recognized for each of the resistance factors of the GFG-model. However, in red stele infested growing areas usually some resistance factors are still effective, either as single factors, or as part of a series (chapter 5). Indeed, *Rpf1* has been effective at the east coast of the USA for more than 30 years (chapter 5). The time period in which their effectiveness will remain depends on the introduction of virulent races from elsewhere, as well as the ease by which the fungus loses avirulence alleles and the ability of the resulting new race to establish itself. Since *P. fragariae* is a soil-borne pathogen, the rate by which virulent races spread will be much slower than for wind-borne pathogens. Sanitary certification of plant stock can further decrease the chance of spreading races. The ability of a new race to establish itself has been suggested to be negatively correlated with the completeness of the involved resistance gene(s) (Parlevliet & Zadoks 1977). In this respect, the moderate resistance of the cultivar Cambridge Favourite may be very valuable.

EARLIER INHERITANCE STUDIES

The occurrence of single major resistance genes for *P. fragariae* was not recognized in earlier inheritance studies. This may have been due to various reasons: lack of appreciation ot incomplete resistance and escapes, variation in disease pressure among experiments leading to variable segregation ratios, and the presence of variable numbers of effective resistance alleles among crosses. Below, some earlier studies are re-evaluated

in view of the GFG-model, taking into account some of the above mentioned potentially disturbing factors.

Stembridge & Scott (1959). These authors examined segregation ratios for resistance in three crosses in a bench test using naturally infested soil. Levels of disease were scored on a scale of 1 (severely diseased) to 5 (no symptoms), of which scores 1, 2, and 3 were previously thought to indicate susceptibility. Considering Midland open pollinated, an SxS cross which felt almost entirely into class 1 (Table 1), class 1 is now thought to indicate susceptibility, whereas class 2 to 5 possess relatively healthy seedlings, which, in the case of the SxS cross, escaped from severe infection. Next, the percentages of resistant seedlings of the other two crosses can be estimated by adjusting the percentage of relatively healthy seedlings (classes 2-5) for escapes as described in chapter 6. For Surecrop x Midland this estimated percentage fitted well with the expectation (Table 1) on the basis of the genotypes of the parents, assuming that each of their resistance genes was effective and present as simplex, and assuming that their genes segregate independently. The cross Gem x Sparkle required the assumption of the presence of a resistance allele in Gem. In a separate test for resistance, Stembridge and Scott (1959) found Gem to be susceptible. This assumption can thus only hold true if the soil sample in which Gem was tested and the sample used in the seedling test differed in their races.

Table 1. Distribution of disease ratings in three crosses as observed by Stembridge & Scott (1959) and a genetic interpretation.

Cross	Number of plants tested	% of seedlings in each resistance class[a]					%resistant seedings		R-genes involved[c]
		1	2	3	4	5	estimated	expected	
Midland Open pollinated	245	94.7	4.9	0.0	0.4	0.0			0 x 0
Surecrop x Midway	238	5.9	5.0	15.5	66.8	6.7	93.8	93.8	1.2.3 x 2?
Gem x Sparkle	231	19.0	20.3	23.4	36.4	0.9	79.2	75.0[b]	?[b] x 2

a: class 1: more than 75% of the root system was infected; class 2: 50%-75%; class 3: 24%-50%; class 4: 1% - 25%; class 5: no symptoms

b: under the assumption that Gem possesses a single effective allele, which allele could not yet be identified.

c: see chapter 5 Tables 1 and 3

Montgomerie (1960). This author assessed segregation ratios for immunity in S_2-crosses of which the parents originated from the selfing of resistant cultivars and selections (Table 2). The data suggest that the resistant S_1-parent from Aberdeen was homozygous for an R-gene, while the resistant S_1 parents from Auchincruive No. 11, Cambridge Vigour, Little Scarlet, *F. ovalis* III, and part of those *F. virginiana* III, and *F. virginiana* VII were simplex. The S_1-descendants of Oberschlesien and part of those of *F. virginiana* III,

and *F. virginiana* VIII gave rise to 26% - 31% symptomless seedlings, which percentages indicate the presence of an incomplete type of resistance due to which only part of the resistant seedlings remained symptomless. These percentages can not be due to the segregation of a recessive allele for resistance since all parents were claimed to be resistant and should thus have been homozygous for this recessive allele.

Table 2. Percentages of healthy seedlings in crosses obtained by two (S_2) or three (S_3) generations of selfing of resistant strawberry genotypes when tested to a single isolate. Data were derived from Montgomerie (1960).

Resistant S_0-parent	Generation	No of seedlings		% of resistant seedlings	
		Tested	Healthy	obtained	expected
Aberdeen	S_2	22	22	100	100
Auchincruive No. 11	S_2	25	17	68	75
Cambridge Vigour	S_2	22	13	59	75
Little Scarlet	S_2	20	13	65	75
F. ovalis III	S_2	21	15	72	75
F. virginiana III	S_2	50	34	68	75
F. virginiana VII	S_2	126	101	80	75
Oberschlesien	S_2	27	7	26	75
F. virginiana III	S_2	180	56	31	75
F. virginiana VII	S_3	26	7	27	75

Van de Weg et al. (1989) I. These authors found the resistance of some North American strawberry genotypes to be single gene based. These genotypes have Md683 as well as Aberdeen in their ancestry, and have been selected for their resistance to a mixture of the isolates A1, A2, A3, A4, and A6. Considering the virulences of these isolates, resistant genotypes should possess *Rpf1*, while *Rpf2* and R3 are not necessary but are allowed to occur (chapter 5). The latter two resistances could not be effective because Cambridge Vigour (R2.3) became diseased on the test field. The firstly observed monogenic resistance for *P. fragariae* should thus have been due to *Rpf1*. The fact that 1:1 segregation ratios were observed despite that only symptomless seedlings were classified as resistant, shows that *Rpf1* was highly effective on this field. Apparently, levels of infection are generally higher with artificial inoculation than with the naturally infection as occurred in this single field experiment.

Van de Weg et al. (1989) II. In this study (chapter 2) the inheritance of the moderate resistance of Cambridge Favourite (MR_{CF}) was examined by testing crosses and selections on a naturally infested field. About 50% of the breeding selections descending from CF

were moderately resistant, which percentage suggested the involvement of a single resistance gene. From the crosses, no conclusion on the number of segregating resistance genes was possible due to the continuous distribution in disease scores. Here, these data are reconsidered. Firstly, a disease severity threshold was assessed based on the cumulative disease distributions of the MR_{CF}xS and SxS crosses (see page 22, fig.1). These differed maximally at rating 7, due to which seedlings rating 8 or 9 are now classified as severely diseased, and those rating 7 or less as relatively healthy. Consequently 47.9% ((62+97)/332 * 100%) of the SxS progenies was severely diseased; the remainder 52.1% is thought to have escaped from severe infection. Of the MR_{CF}xS crosses, 77.6% was relatively healthy. By adjusting this percentage for escapes (chapter 6) and by assuming that only a few or no seedlings with the CF-resistance were severely diseased, the senior author now estimates the percentage of seedlings possessing this resistance to be 53%. This percentage fits with the monogenic inheritance as expected from the proportion of moderately resistant selections. This newly identified resistance gene has not yet been included in the GFG-model.

The present approach for estimating the proportion of resistant seedlings of an RxS cross is mathematically equivalent to determining the reduction in the proportion of severely diseased seedlings of an RxS cross relative to that of an SxS cross (e.g. (0.479-0.223) / 0.479 = 0.52). It is interesting to note that this approach has also been helpful for the recognition of a resistance gene which confers a similar level of moderate resistance in the apple cultivar Merton Worcester to powdery mildew caused by *Podosphaera leucotricha* (Van de Weg & Janse 1992).

The foregoing examples illustrate that the presence of various individual resistance genes can be established in earlier inheritance studies. This once more shows the power of the model as well as the quantitative approaches in the analyses of the data. Of future interest remains those data which could not yet be explained by the current model (e.g. Stembridge 1961; Mussel & Fay 1971; Galletta et al. 1989), since these data may indicate the existence of new resistance genes.

All crosses examined in this and the previous chapters showed 1:1 segregation ratios for the effective resistance genes, except the S_2 cross of Aberdeen in which a 1:0 ratio was observed (Table 2). This predominant occurrence of 1:1 ratios indicates that the relative genes were generally present as simplex. This is not surprising since most of the resistant parents arose themselves from an RxS cross which favours the occurrence of simplex genotypes, independently of the ploidy level at which a gene segregates.

COMPARISONS WITH OTHER GFG-MODELS

Number of genes. The number of resistance and avirulence factors of the proposed model is low compared to that for other, more extensively examined pathosystems. Considering resistances for oomycetes, 11 race specific R-genes were recognized in wild potato species for resistance to *Phytophthora infestans* (Schick R, Schick 1961; Malcolmson & Black 1966; Colon & Budding 1993), and 30 race specific R-factors in lettuce for resistance to *Bremia lactucae* (Bonnier et al. 1994). The numbers of race specific R-genes in cereals for resistance to rust diseases are also considerable, e.g. 30 in flax to *Melampsora lini* (Lawrence 1988). Extension of the series of examined strawberry cultivars and *P. fragariae* isolates will therefore most likely lead to the identification of additional resistances.

Incompleteness of resistance. Major genes for incomplete resistance occur in various other GFG-relationships, including those of the rust and mildew diseases in cereals and coffee, and that for apple-scab (Hough et al. 1953; Moseman et al. 1965; Hooker 1966; Eskes 1982; Crute 1985). Incomplete resistance is usually distinguished from susceptibility and complete resistance by the predominant infection type, due to which disease severity can be scored on an ordinal scale. Incomplete resistance results in restricted sporulation or a slight to moderate mycelial development in a few lesions, whereas complete resistance and susceptibility allow respectively no sporulation or abundant sporulation (or mycelial development) of many lesions. Only in a few relationships incomplete, race specific resistance has been recognized by means of quantitative disease assessments of a single parameter, including wheat-*Ustilago tritici* (Oort 1944), potato-*Globodera rostochiensis* (Janssen et al. 1991), and strawberry-*P. fragariae* (this thesis), of which only the latter evaluated the degree of colonization of the host.

Also in other host-pathogen systems, incomplete resistance sometimes resulted in divergent segregation ratios for resistance among researches due to differences in resistance classification. In case of the widely explored *Vf* gen in apple, which confers resistance to scab, seedlings are sometimes only classified as resistant if they did not sporulate at all (Kellerhals 1989), while in other studies restricted sporulation on some lesions is allowed (Hough et al. 1953). Failure to appreciate incomplete resistance might also explain the unexpected occurrence of resistant seedlings from presumed SxS crosses (Skinner & Stuteville 1985) or deviations between observed and expected percentages of resistant seedlings in RxS and RxR crosses (Bonnier et al. 1992).

Environmental effects. The expression of R-genes, including some of the genes to the

oomycetes *Bremia lactucae* and *Phytophthora megasperma*, is in various cases sensitive to allele dosages, genetic background, and experimental conditions such as temperature, light intensity, and day length, which circumstances can effect the outcome of a GFG-analysis of host-pathogen interactions (Walker 1965; Ward & Lazarovits 1982; Browder 1985; Keeling 1985; Judelson & Michelmore 1992). In strawberry, non of the fore going factors have yet been examined, but soil pH was shown to have a differential effect on resistance expression (Maas 1976).

Specialization of the pathogen. Most pathogens which exhibit a GFG-relationship have a very restricted host range. In this respect, *Phytophthoras* might be somewhat departing since *P. fragariae* var. *fragariae* not only infects all *Fragaria* species, but also several other genera of the Rosacea family, including *Dryas*, *Geum*, *Potentilla*, and *Rubus* (McKeen 1958; Moore et al. 1964; Converse & Moore 1966; Pepin 1967), while *P. infestans* infects *Solanum* as well as *Lycopersicon* species (Wilson & Gallegly 1955).

Earlier observations suggest the presence of GFG-relationships between *P. fragariae* and at least some of its other hosts. Firstly, race-specific resistance was observed within *Geum* and *Potentilla* (Pepin 1967). Secondly, segregation ratios reported by Moore et al. (1964) allow the presence of monogenic, incomplete resistances in *Potentilla* species (see Table 3). However, definite conclusions on the genetics of these resistances could not be drawn because of the small size of the crosses as well as lack of information of the parentage of the crosses.

Finally, also raspberry-*P. fragariae* var. *rubi* may exhibit a GFG-relationship since here too resistance is race specific (Kennedy & Duncan 1993b).

Table 3. Number of resistant and susceptible seedlings descending from four *Potentilla* species and one subspecies, when tested with isolate A5 of *P. fragariae* var. *fragariae*. Data were derived from Moore et al. (1964).

Species	Number of seedlings		% Resistant seedlings	χ^2-value	
	Resistant	Susceptible		1:1	3:1
P. andicola	22	39	36%	4.7 **[b]	
P. rupestris	21	38	36%	4.9 **	
Potentilla sp.	17	25	40%	1.5 * **	
P. glandulosa	26	31	41%	0.4 * **	
P. rupestris var. *villosa*	36	18	67%	6.0 **	2.0 * **
Cultivated strawberry[a]	0	65			

a: Cultivar Blakemore & Midland x self seedlings

b: *, ** Not significantly different from the expected ratio at respectively the 99% and 95% confidence level according to the χ_1^2-test.

BREEDING

Relevance of the GFG-model. The proposed GFG-model is a major step in the elucidation of the genetics of resistance to *P. fragariae*. It can be highly valuable for resistance breeding programs in that it allows the combining of consciously chosen resistance genes. Since it also allows a well-founded choice of fungal isolates to which strawberry genotypes have to be tested to establish the presence of the desired resistances. The model has therefore been fully integrated in the red stele breeding program at CPRO-DLO. The first triple resistant cultivar from this program will be released soon and was bred by Meulenbroek & Van de Lindeloof.

Constraint: lack of knowledge on the prevalent races. In Europe, breeding for resistance to red stele is still impeded by lack of insight in the occurrence and dispersion of races, due to which breeders can not decide on the required resistance genes. This contrasts with the situation for Eastern North America, where breeders took the benefit of such knowledge for decades. European research on this topic is essential and should be initiated preferably in the very near future. The proposed GFG-model will be of help to determine which strawberry genotypes should be used as differentials, while the extensive isolate collection of Kennedy and Duncan (1993a) can provide a good starting point for the series of isolates to be tested.

Molecular markers for avirulence genes would, if available, greatly facilitate the classification of isolates into races. Reducing the costs of race identification, they would allow diagnostics to be payable for growers, and, for the benefit of breeders, allow a continuous monitoring of the dispersion of races. Putative linked markers can be assessed by the molecular testing of a series of isolates with known virulences. Such research has been initiated by IPO-DLO in the Netherlands jointly with the Scottish Crop Research Institute (Bonants & Duncan, personal communication).

Challenges: Molecular markers for resistance. The availability of molecular markers for resistance could further improve the breeding efficiency. Tests based on such markers are fast, reliable, can be performed throughout the year, and do not suffer from epistatic effects among resistance genes facilitating the pyramiding of these genes. In research connected to this thesis, such markers have been identified for the *Rpf1* gene. The two nearest flanking markers were at 1.6 and 3.3 cM from the gene (Haymes et al. 1997a, b), and their linkage to the gene was shown to be conserved in many cultivars and advanced breeding selections (Haymes et al. 1997c). They can therefore be used in marker assisted breeding. However, to take the full advantage of this approach, such markers should be

available for each of the desired resistance genes.

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SAMENVATTING

Roodwortelrot is veelal de belangrijkste bodemziekte van aardbei (*Fragaria spp.*) in gebieden met koele, vochtige gronden. Soms trad deze ziekte zo heftig op, dat de teelt van aardbei in een gebied gestopt werd. Roodwortelrot wordt veroorzaakt door de schimmel *Phytophthora fragariae* var. *fragariae*. Het is in Europa een quarantaine ziekte met een nul-tolerantie. Dit betekent dat een partij planten waarin deze ziekte wordt geconstateerd, niet mag worden verhandeld. Het perceel waar de besmette planten stonden mag nooit meer gebruikt worden voor de vermeerdering van handelsmateriaal. Roodwortelrot is dus een bedreiging voor zowel telers als vermeerderaars.

Resistente rassen. Het telen van resistente rassen is een natuurlijk alternatief voor het gebruik van chemische bestrijdingsmiddelen. Deze benadering is in Noord Amerika effectief gebleken bij het bestrijden van roodwortelrot. In West Europa zijn tot op heden geen resistente rassen beschikbaar. In Nederland is in 1968 het DLO Centrum voor Planten Veredeling- en Reproduktie Onderzoek (CPRO-DLO) te Wageningen begonnen met het veredelen op roodwortelrotresistentie. Bij de aanvang van dit onderzoek in 1990 waren er een aantal vergevorderde resistente selecties verkregen. Omdat hun teeltwaarde te veel achter bleef bij die van het CPRO-DLO ras Elsanta, dat in Nederland een marktaandeel van 95% heeft, is geen van deze selecties als ras in de handel gebracht.

Het veredelen op resistentie werd tot dusver bemoeilijkt door de aanwezigheid van fysio's van de schimmel, door het ontberen van een betrouwbare resistentie-toets, èn door het ontbreken van kennis over de genetische basis van resistentie. Het onderzoek dat in deze dissertatie beschreven wordt had tot doel om de twee laatst genoemde problemen te overwinnen, en zo de mogelijkheden te vergroten tot het maken van elite, roodwortelrotresistente aardbeirassen.

Fysio's van de schimmel. Er bestaan verschillende vormen van de schimmel, welke fysio's genoemd worden. Deze fysio's onderscheiden zich door de aardbeirassen die ze kunnen aantasten. Omgekeerd geldt dat de resistentie van een ras slechts effectief is tegen een aantal fysio's. Er zijn verschillende resistenties in aardbei bekend. Welke resistenties een ras moet hebben om vrij van roodwortelrot te blijven, hangt af van de fysio's die in een teeltgebied voorkomen. Soms kan met één resistentie worden volstaan, in andere gevallen moeten diverse resistenties tegelijk aanwezig zijn. Voor de veredeling is het dus nodig om te weten welke fysio's aanwezig zijn in het teeltgebied. Daarnaast is het belangrijk om over een methode te kunnen beschikken waarmee vastgesteld kan worden welke (combinaties van) resistenties effectief zijn. Dit onderzoek voorziet in zo'n methode middels de ontwikkelde resistentietoetsen en middels het ontwikkelde genetische model.

Resistentie-toets. Plantentoetsen zijn ontwikkeld om de resistentie van aardbeirassen en kruisingsnakomelingen vast te stellen. Deze toetsen vinden plaats onder geconditioneerde experimentele omstandigheden. Hun belangrijkste vernieuwing is de systematische manier waarmee ze rekening houden met onvolledige resistentie. Dit wordt bereikt door het aantastingsniveau van het onderzochte genotype te vergelijken met dat van een universeel vatbaar ras. Het onderzochte genotype heeft resistentie indien het significant minder is aangetast dan het referentieras. Hiermee maken deze toetsen in feite gebruik van een *flexibele* aantastingsdrempel voor het onderscheiden van resistente en vatbare rassen. Hiermee onderscheiden ze zich van eerdere toetsen, waarin steeds een vast, veelal willekeurig gekozen aantastingsniveau gebruikt werd.
Een andere vernieuwing heeft betrekking op het vaststellen van uitsplitsingsverhoudingen voor resistentie en vatbaarheid in overervingsstudies. De gebruikte methode houdt rekening met het verschijnsel dat genetisch vatbare nakomelingen soms niet of weinig aantasting te zien geven doordat ze aan (zware) infectie ontsnappen.
Deze twee vernieuwingen leidden tot de identificatie van enkele individuele resistentiegenen.

Genetica van resistentie. Er is een genetisch model ontwikkeld. Dit model bestaat uit vijf resistentiefactoren in aardbei, en vijf complementaire avirulentiefactoren in de schimmel. De resistentie van een aardbeiras voor bepaalde fysio's kan nu verklaard worden door de interactie tussen deze resistentie-en avirulentiefactoren. Dit zogenaamde gen-om-gen model verklaart de resistentie van een aardbeiras voor de verschillende fysio's van de schimmel. Het model is gelijkvormig aan het gen-om-gen model dat in de jaren veertig en vijftig door Flor ontwikkeld is voor de relatie tussen vlas en *Melampsora lini*, de veroorzaker van vlasroest. Overervingsstudies aan twee van de vijf resistentie-factoren toonden aan dat elk van deze factoren op de werking van één gen berust. De betreffende genen zijn benoemd als *Rpf1* en *Rpf2*. Hiermee heeft dit onderzoek aangetoond dat fysiospecifieke resistentie voor *P. fragariae* een monogene eigenschap is in aardbei. Het beschreven model is het eerste gen-om-gen model voor een bodemschimmel, en de hier beschreven genen zijn de eerste geïdentificeerde resistentiegenen in aardbei.

Het vaststellen van een gen-om-gen relatie is een grote vooruitgang in het opheldéren van de genetica van resistentie voor roodwortelrot. Het model biedt goede mogelijkheden om de efficiëntie van de veredeling te verhogen. Het maakt het mogelijk om bewust gekozen resistentie-genen samen te brengen in nieuwe rassen. Het model maakt het ook mogelijk om een universeel bruikbare set van toets-rassen samen te stellen. Zo'n set is essentieel om fysio's van de schimmel effectief te kunnen onderscheiden. Hierdoor kunnen de resultaten van dit onderzoek bijdragen aan een betrouwbaarder, rendabeler en milieuvriendelijker teelt van aardbei in gebieden met roodwortelrot.

NAWOORD

Het experimentele onderzoek dat in de hoofdstukken 3 tot en met 7 beschreven wordt heeft plaatsgevonden in de periode 1990-1994 bij het voormalige Instituut voor de Veredeling van Tuinbouwgewassen (IVT) en het latere DLO-Centrum voor Plantenveredelings- en Reproduktie Onderzoek (CPRO-DLO). Hieronder wil ik enkele mensen met name noemen die met mij betrokken zijn geweest bij het tot stand komen van het proefschrift.

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CURRICULUM VITAE

The author was born on 9 June 1958 in Dordrecht, The Netherlands. In 1976 he started his studies in Plant Breeding at the Agricultural University in Wageningen (LUW). He graduated in 1984 with *Plant Breeding* and *Plant Physiology* as main subjects and *Economics* as minor subject. During his study he has been a member of the faculty board of the LUW and participated actively in the reorganisation of the educational programs. As part of his education, the author participated in research on the genetics of resistance to *Hemileia vastatrix* in coffee at the Instituto Agronômico of Campinas, Brazil, as well as to *Puccinia hordei* in barley at the LUW as a student of Dr. J.E. Parlevliet.
In 1985 the author started as a researcher at the Institute of Horticultural Plant Breeding (IVT) which is now merged in DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO). His current position is scientist for genetics and plant pathology in the Department of Vegetable and Fruit Crops. Here he has been responsible for research on the resistance to *Phytophthora fragariae* in strawberry and to *Nectria galligena* and *Podosphaera leucotricha* in apple.