2019-2022

ISF Passalora fulva project tomato

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ISF DRT WG

2019-2022

Project for characterisation of existing and new strains and validation of a new differential set for *Passalora fulva* (Pf) – Tomato

Introduction:

In tomato many resistance genes for *Passalora fulva* exist. Since 1974 the research group of Pierre de Wit has studied the pathosystem *Cladosporium fulvum*-tomato and has cloned several avirulence (Avr) genes of the fungus (de Wit, 2016).

The current name of the disease has changed from *Fulvia fulva* to *Passalora fulva* but *Cladosporium fulvum* and *Fulvia fulva* are still in use. The resistance genes (Cf-genes) take their name from the old name of the pathogen. This is also the name that most breeders use in their selling guides.

The interaction between *Passalora fulva* and tomato typically complies with the "gene-for-gene" model, in which Avrs of the fungus are specifically recognised by matching Cf resistance proteins of the plant. When only non-matching Avr/Cf combinations are present, the fungus will be able to colonise the host tissues and will cause disease. In the case of one or more matching Avr/Cf combinations being present, the Cf protein will perceive the Avr and a defence response will be mounted. Defence is typically associated with a hypersensitive response (HR), which culminates in a localised programmed cell death and prevents the pathogen from further proliferation.
Most of the Cf genes matching the Avr genes of *Passalora fulva* have been cloned.

Not all Cf genes are used in commercial varieties. The most commonly used ones are: Cf-2, Cf-4, Cf-5 and Cf-9. Cf-9 seems to be particularly effective as races overcoming it (e.g. race 2.5.9) have not spread significantly in the last 15 years.

Passalora fulva races have been named using a complex scientific system and Hubbeling (1978) introduced a system of grouping them. This system is also used by the international Union for the Protection Of new Varieties (UPOV) and the European Community Plant variety Protection Office (CPVO), for testing tomato varieties, as it is based on the most commonly used genes (see Annex).
The correspondence between the races and race groups is indicated in the table 1 below.

	Race →	0*	1	2*	3	4*	1.2.4	2.4*	5*	2.3.4.5	2.4*	2.5*	2.4.5*	2.5.9*	2.4.5.9	1.2.3
	Group →			A		B	C		D				E			
Variety	Resistance Gene															
- Monalbo - Moneymaker - Motelle	none	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
- Stirling Castle - Leaf Mould Resister	*Cf-1*	R	S	R	R	R	S	R	R	R	R					S
Vetomold	*Cf-2*	R	R	S	R	R	S	S	R	S	S	S	S	S	S	S
V121	*Cf-3*	R	R	R	S	R	R	R	R[1]	S	R	R	R	R	R	S
Purdue 135	*Cf-4*	R	R	R	R	S	S	S	R	S	S	R	S	R	S	R
- IVT 1149 - PI 187002-1 - Ontario 7717	*Cf-5*	R	R	R	R	R	R	R	S	S	R	S	S	S	S	R
- F77-38 (also called Ontario 7818)	*Cf-6*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
- IVT 1154 - Ontario 7719	*Cf-9*	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R
Vagabond	*Cf-2, Cf-4*	R	R	R	R	R	S	S	R	S	S	R	S	R	S	R
- F1 Vetomold x IVT 1149	*Cf-2, Cf-5*	R	R	R	R	R	R	R	R	S	R	S	S	S	S	R
- F1 Vagabond x IVT 1149	*Cf-2, Cf-4, Cf-5*	R	R	R	R	R	R	R	R	S	R	R	S	R	S	R
- F1 Vagabond x Ontario 7719	*Cf-2, Cf-4, Cf-9*	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R

Note: * Races most frequently used by breeders; [1] resistance is partial

Table 1. Differential table from ISF website

In table 2 an alternative coding system for resistances used by many companies is presented. It corresponds to the Hubbeling groups, races and genes from table 1. As in the meantime as mentioned above the name and coding for the disease has changed from Fulvia fulva to Passalora fulva, the old code Ff is still used.

Codes used by companies		**Races** (see annex for complete set)	**Genes**	**ISF Code[1]**
Group Identifier	**Group(s)**			
C1	A	2	*Cf-1*	Ff: 2
Cbd	B, D	4 and 5	*Cf-2*	Ff: 2,4
C2	A, B, D	2, 4 and 5	*Cf-2, Cf-4*	Ff: 2, 4, 5
C3	A, B, C	1, 4, 1.2.4 and 2.4	*Cf-5*	Ff: 2, 4, 1.2.4, 2.4
C4	A, B, C, D	2, 4, 1.2.4, 2.4 and 5	*Cf-2, Cf-4, Cf-5*	Ff: 2, 4, 1.2.4, 2.4, 5
C5	A, B, C, D, E	2, 4, 1.2.4, 2.4, 5 and 2.4.5	*Cf-9*	Ff: 2, 4, 1.2.4, 2.4, 5, 2.4.5

Table 2. Alternative coding system according to the ISF website

This system, which is used quite commonly (however not always), is very simple and appreciated by companies for use in the marketing of their varieties by easy mentioning it in catalogues and on seed packages, esp. for Cbd and C5 which appear relatively frequently.

Introduction of different Cf genes by breeders has successfully protected commercial tomato cultivars during the last decades.
However, for many years already, new strains have been detected which attack previously resistant varieties with Cf-9 or other resistant genes and cannot be classified according to the

system mentioned in table 1.
Especially at organic growers who grow tomato at lower temperature, these new strains are found. Some companies have already bred varieties with new genetics, giving resistance to these new strains.
The new strains are now part of a new collection that has been built by Pierre de Wit (Laboratory of Phytopathology, WUR), but the strains have only been partially characterized.

Strikingly, it also appeared that several new strains of the fungus can overcome the Cf-9 gene only, and not the genes Cf-2 Cf-4, Cf-5 and/or Cf-6 (Iida et al., 2010), suggesting that some of the new commercial tomato cultivars contained the Cf-9 gene only. It also appeared in 2015 (Iida et al. 2015) that in Japan Cf-6 was still not broken.
This shows that some of the currently employed tomato cultivars are prone to infection by *Passalora fulva* and that a new set of tomato differentials is required for the breeders to characterize the resistance of their cultivars to *Passalora fulva*.

The ISF DRT WG decided to clarify the present situation with unknown new strains and new genetics in this project. ISF has developed coding guidelines for the denomination of races of diseases. For *Passalora fulva* an objective, clear and durable race coding and denomination system of present and new races that are relevant for the tomato market, which corresponds to these coding guidelines, should be developed in this project.
Companies prefer to develop a simple coding system with codes and groups like in the system shown in table 2 above, as it is very easy and recognisable in the use in commercial catalogues and on packages compared to the complex scientific system. But it is unclear and has to be seeked out on the basis of the outcome of the first part of the project, if the simple group system with groups A to E as now in use, can be further extended with new isolates and differentials.

Another way of setting up a durable system which is easily understandable to seed industry players and also for the growers, could be according to the system that is developed by IBEB for *Bremia lactucae* in lettuce. This is shown in the table 3 below.

	GreenTowers	Dandie	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	FrRsal-1	Argelès	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille	C sextet code
		Dm3	Dm4	Dm14	Dm15	Dm16	Dm18	Rsal-1	R38								
Set position		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	
Sextet value		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	
BI: 5US	+	+	-	+													
BI: 6US	+	+	+	+													
BI: 7US	+	+	-	+	+	+	+		-	-	-	(+)	-	-			61-.-.
BI: 8US	+	+	+	+		+	+		-	-	-	(+)	-	-			
BI: 9US	+	+	-	+	+	+	+	+	-	-	+	(+)	-	-	+	-	61-25-02
BI: 16EU	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	19-00-00
BI: 17EU	+	+	-	+	+	-	+	+	-	-	-	(+)	-	-	-	-	45-17-00
BI: 18EU	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	50-00-00
BI: 20EU	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	51-00-00
BI: 21EU	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	27-01-00
BI: 22EU	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-	-	46-32-00
BI: 23EU	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	19-02-00
BI: 24EU	+	-	+	-	-	+	+	-	+	-	-	-	-	-	(-)	-	50-02-00
BI: 25EU	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	50-01-00
BI: 26EU	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	51-03-00
BI: 27EU	+	+	+	+	+	-	+	-	+	+	-	-	+	-	-	-	47-38-00
BI: 29EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	62-07-00
BI: 30EU	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-	46-06-02
BI: 31EU	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	39-12-02
BI: 32EU	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	+	27-00-04
BI: 33EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	62-07-06
BI: 34EU	+	-	+	+	-	+	+	+	+	+	+	-	-	+	(-)	-	54-15-01
BI: 35EU	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	62-15-06

Legend: (+) indicates slightly reduced sporulation, (-) indicates no sporulation with necrosis or very weak sporulation, as defined in the harmonized scale given in Fig. 1.

Note: BI: 19EU and BI: 28EU are nonexistent. Resistance claims for BI: 16-35EU do not apply to the nonexistent isolates.

Table 3. IBEB differential table for Bremia isolates in lettuce

The IBEB C set of 15 differential varieties consists of two groups of six varieties (sextets) and one group of three varieties. The position of a differential within the group determines the sextet value of that differential. Sextet values are ascending powers of 2 (1, 2, 4, 8, 16 or 32). The sextet code of an isolate is the sum of the sextet values of the differentials that are susceptible, as indicated by + or (+) in the table.

With a comparable system to be developed in this project for *Passalora fulva* in tomato, one can immediately deduct the tomato lines of the differential set on which the race of interest is virulent. Presently, at the Laboratory of Phytopathology, WUR, a differential set of five lines is used, only allowing to identify a limited number of races, as more complex races cannot be discriminated.

Not only for claims in the market, all companies should use the same resistance terminology and use the same differential sets in tests with the disease. It is also in the interest of DUS testing in tomato to clarify the situation with new genetics and isolates for the use of the characteristic “resistance to *Passalora fulva”*.

In a former project proposal from WUR, it was the objective to characterize this complete collection. However, it was decided by the ISF DRT WG not to carry out this project proposal. Instead it was proposed to carry out this ISF project for the characterization of new and old *Passalora fulva* strains and establishing a new differential set and race denomination system.

Different steps in this project:

1. Definition of a differential set needed for the characterisation of available isolates

2. Inventory and characterization of available isolates

3. Definition of a set of maximum 20 different isolates

4. Ring test with a set of old and new isolates on a differential set of varieties

5. Development of a new set of tomato differentials carrying commonly and not commonly used Cf-genes

6. Definition of a new coding system

7. Publication of the results

Step 1. Definition of a differential set needed for the characterisation of available isolates

It was decided that the differential set of varieties would consist of a part of the differential sets of ISF (shown in the introduction).
The *Passalora fulva* part of the ESA harmonized resistance table of tomato is part of the ISF differential set and does not provide extra varieties.

From the ISF differential table only the varieties with different Cf-genes, mentioned in table 4 below, from which seed is available through the MATREF collection, were used.

Variety	Resistance gene
Monalbo	None
Stirling Castle	Cf-1
Vétomold	Cf-2
V121	Cf-3
Purdue 135	Cf-4
IVT 1149	Cf-5
Ontario 7818	Cf-6
IVT 1154	Cf-9

Table 4. Differentials from ISF differential table used in this project

From literature it was known that also Cf genes Cf-8 and Cf-11 exist. However Cf-8 is allelic with Cf-4 (Gerlagh, 1989) and sometimes even proposed to be the same. Cf-11 is of very limited value because races can easily evolve to include virulence for this gene (O'Neill, 2013). Therefore both genes are not commonly used. It is known that Ontario 7716 contains Cf4 and Cf-11 (Enya, 2009). It was decided not to add this variety, since no seeds were available.

Also varieties or lines with Cf genes different from the above mentioned genes were added. In the kick-off meeting of 3 December 2019 (by WebEx), it was decided to add the varieties Chelino from ENZA Zaden which is known to have novel Cf genes (unknown which), and of which a seedlot in larger quantities was already available at Naktuinbouw for the ring test. It was decided not to include the variety Claudino, which has the same genetics.
Also the variety Completo from Bayer which was known to contain also a novel Cf-gene was added as candidate differential.

Step 2. Inventory and characterization of available isolates

It was decided that the initial collection of isolates to be inventoried, would include the existing reference isolates from the Naktuinbouw – Plantum isolate collection (races A, B, C, D and E) and race 0 and the race 2.4.5 from group E from the MATREF collection.
Furthermore participating companies would provide new isolates to a maximum of 5 per participating company, which were thought to behave different from the existing defined isolates and had broken known resistance genes. Eventually more than 5 isolates could be provided, since not all participants were expected to propose candidate isolates.
It was preferred that as much as possible isolates from different tomato growing areas in the world (e.g. NW Europa, Japan, Canada) where *Passalora fulva* is a problem were provided.

From this initial collection of isolates, the virulence spectrum should be determined (as far as not already known yet) by the laboratories of the participant who collected them.

Tests would be carried out on at least 12 plants of each of the 8 differentials.
For the existing reference isolates, Naktuinbouw would characterise the reaction from races A, B, C, D and E from the Naktuinbouw – Plantum isolate collection. GEVES would do the same for race 0 and the race 2.4.5 from group E from the MATREF collection.

It was decided that the seeds of the differentials used by the participants would be the normal seeds available in the relevant labs. However if a lab did not have available seeds of these differentials, it could contact the coordinator of the project for seeds.
In the meeting of 3 December 2019 the needs of participants for seeds and/or isolates were discussed.

Seeds from the MATREF collection would be sent directly by GEVES.
Seeds of Chelino and Completo would be sent by Naktuinbouw.

For sending seeds to participating labs MUA's were prepared.
Since the MUA to be prepared for ISF projects (on the basis of the MUA used by ISHI) was almost ready, there was general agreement to use that MUA.
Naktuinbouw prepared both the Framework agreement for general use and the Standard agreement for this project and sent around for signing and collected the signed Standards agreements. ISF collected the signed Framework agreements.

Companies tested their isolates and the results were delivered in the initial characterization table (with year, origin, date of test and results on a the differential set).
As also results of not very recent tests could be used, an indication of the moment of testing was added.
All participants should include in the initial characterisation table as much as possible results on the indicated differentials. If it was not possible to provide results on the indicated differentials but other differentials were used, the genetic background of those differentials was indicated.
The focus of the initial characterisation was on Cf-9 breaker isolates, since almost all problems are related to Cf-9 varieties grown in areas where *Passalora fulva* is a problem.

The deadline for sending this information was end of February 2020, but due to the delay in preparation of MUA's and sending the samples and the start of the covid pandemic, the last results were delivered later.

Step 3. Definition of a set of maximum 20 different isolates

Also due to the covid pandemic, the results of the initial characterisation were not discussed in a face to face meeting in Madrid as planned, but in an online meeting on 22 April 2020.

<u>Results of initial characterisation</u>

Results of 15 Cf-9 breaking isolates which had been characterized for its virulence spectrum were sent in by 5 companies, Bayer, ENZA, Gautier, Rijk Zwaan and Sakata JP.

Results are presented in table 5 (for Cf-9 breaking isolates) and table 6 (for standard reference isolates). Results of the Cf-9 breaking isolates are sorted on the binary code of the isolates obtained through the sum of S (blancs are also counted as S) which gives an indication of similarity of the isolates despite missing information. As indicated in the footnotes, in some cases other differentials than the predefined ones had been used.

Isolate #	Company	Collection date	Country collected	date of test	Monalbo	Stirling Castle	Vétomold	V121	Purdue 135	IVT 1149	Ontario 7818	IVT 1154	Chelino	Completo	Q	Binary	Include in ring test	Reason for choice/skip
					no Cf	Cf-1	Cf-2	Cf-3	Cf-4	Cf-5	Cf-6	Cf-9						
					1	2	4	8	16	32	64	128	256	512				
AB187	Enza	27-5-2014	South-Korea	March 2020	S	S**	S	S	R	R	S	S	S	S	1	975	yes	unique
TS-20	Bayer	various	Switzerland	various	S*	nt	S*	S	HR*	HR*	nt	S*	HR	IR****	0	207	no	probably same as TS-36 and TS-38
TS-30	Bayer	various	Germany	various	S*	nt	S*	S	HR*	HR*	nt	S*	HR	IR****	0	207	no	probably same as TS-36 and TS-38
TS-34	Bayer	various	Germany	various	S*	nt	S*	S	HR*	HR*	nt	S*	HR	IR****	0	207	no	probably same as TS-36 and TS-38
TS-36	Bayer	various	UK	various	S*	nt	S*	S	HR*	HR*	nt	S*	HR	IR****	0	207	yes	most recent isolate from Bayer
TS-38	Bayer	various	France	various	S*	nt	S*	S	HR*	HR*	nt	S*	HR	IR****	0	207	yes	most recent isolate from Bayer
Ff-86	Sakata JP	16-dec-14	Japan	April 2020	S	S	HR	S	S	S	HR	S	HR	HR	1	187	yes	unique
Ff-77	Sakata JP	12-aug-11	Japan	April 2020	S	S	HR	S	S	HR	HR	S	HR	HR	1	152	yes	unique
RZ 2	Rijk Zwaan	2-3-2009	Japan	various	S*		R*		S*	R*	R*	S*			0	152 (920)	no	probably same as Ff-77
U146	Enza	10-8-2011	Netherlands NB	March 2020	S	IR**	S	S	R	R	R	S	R	R	1	151	yes	probably unique (but as IR could be S as indicated in the meeting, probably same as Ff-72))
Ff-72	Sakata JP	9-jun-11	Japan	April 2020	S	S	S	S	IR****	HR	HR	S	HR	HR	1	143	yes	probably same as TS-36 and TS-38, but different region
Ff-87	Sakata JP	13-jul-16	Japan	April 2020	S	S	S	S	HR	HR	HR	S	HR	HR	1	143	no	probably same as Ff-72
G18.035	Gautier	sep-18	France dpt 44 w	30-3-2020	S	S	S	S	R	R	R	S	R***	R****	1	143	yes	probably same as TS-36, TS-38 and Ff-72, but different region
DE1225	Rijk Zwaan	20-6-2012	Germany	various	S*		S*		R*	R*	R*	S*			0	143 (911)	yes	probably same as TS-36, TS-38 and Ff-72, but different region
Ff-80	Sakata JP	30-jan-12	Japan	April 2020	S	S	HR	S	IR****	HR	HR	S	HR	HR	1	139	yes	unique

*isogenic Cf-lines of Moneymaker are used in test (instead of Monalbo, Vétomold, Purdue 135 and IVT 1154)

** Leaf mould Resister is used in test (instead of Stirling Castle), level of resistance between S and IR

*** Yellowish spot without sporulation (in an previous test more agressive, I had localized sporulation)

**** Yellowish spot without sporulation

Binary: sum of S (blancs are also counted as S)

Table 5. Results of initial characterisation of Cf-9 breaking isolates

Isolate #	Company	Collection date	Country collected	date of test	Monalbo	Stirling Castle	Vétomold	V121	Purdue 135	IVT 1149	Ontario 7818	IVT 1154	Chelino	Completo	Q	Binary	Conclusion
					no Cf	Cf-1	Cf-2	Cf-3	Cf-4	Cf-5	Cf-6	Cf-9					
					1	2	4	8	16	32	64	128	256	512			
race A	Bayer	WUR-phytopathology			S	nt	S	nt	HR	HR	nt	HR	nt	HR	0	5 (79)	
race B	Bayer	WUR-phytopathology			S	nt	HR	nt	S	HR	nt	HR	nt	HR	0	17 (91)	
race C	Bayer	WUR-phytopathology			S	nt	S	nt	S	HR	nt	HR	nt	HR	0	21/23 (31)	
race D	Bayer	WUR-phytopathology			S	nt	HR	nt	HR	S	nt	HR	nt	HR	0	33 (107)	
race E	Bayer	WUR-phytopathology			S	nt	S	nt	S	S	nt	HR	HR	HR	0	55 (63)	
MATREF 04-01-03-02 PAS 633 Expected race 2.4.5	GEVES	5-9-2003	INRA Montfavet Name: Nantes 89 V	2-4-2020	S	S	S	S	S	S	R	R	R	R	1	63	Expected race 2.4.5, observed 1.2.3.4.5 This isolate has probably this comportement since we obtained it.
MATREF 04-01-03-01-03 PAS 632 Expected race 0	GEVES	13-6-2007	MATREF partner	2-4-2020	S	S	R	S	S	R	R	R	R	R	1	27	Expected race 0, observed 1.3.4 We have doubt concerning the evolution of our strain expected race 0. As soon as it will be possible, we will characterize again this strain using another date of conservation.

Table 6. Results initial characterisation of standard reference isolates

There were some missing results because of different reasons.
Bayer did not have test results on Stirling Castle and Ontario 7818 because of no germination of seeds.
Rijk Zwaan was not able to carry out the tests but gathered information about previous tests. Results of some differentials was therefore not available.
Both companies did not test with the standard differentials but with isogenic lines of Moneymaker.
Is was also agreed that both companies would still carry out the missing tests as soon as it is possible according to corona and seasonal reasons.
Also Sakata was still carrying out some last tests and sent in results shortly after the meeting. These are also included in table 5 and did not lead to a change in decisions after the meeting.

Regarding the standard isolates tested by GEVES and Naktuinbouw some problems were encountered.
Race 0 of MATREF was observed 1.3.4. GEVES had doubts concerning the evolution of their strain expected race 0. As soon as it will be possible, they will characterize again this strain using another date of conservation and report as soon as results are available.
Race 2.4.5 of MATREF was observed 1.2.3.4.5. GEVES indicated this isolate has probably this constitution since they obtained it and is in that case probably the same as race E.
Test of isolates A to E were not carried out by Naktuinbouw, but Bayer helped with providing results of previous tests with these same isolates (thanks Ton!). Therefore some results were missing at the time of the meeting.
Naktuinbouw carried out the tests on all standard differentials shortly after the meeting but it did not change any of the conclusions or decisions.

Selection of isolates for ringtest

During the meeting the results were discussed. And although not all results of characterisation were available, out of the 15 characterised Cf-9 breaking isolates, 10 isolates were chosen for inclusion in the ringtest.
Some isolates appeared to be for sure unique, others probably the same but were chosen because of the predefined selection criterion of origin from different growing areas of tomato.
The reason for choice or no choice is indicated in the last column of Table 5.
Despite some problems encountered in the characterisation of the 7 standard reference isolates, it was decided to include all these in the ringtest.

Step 4. Ring test with a set of old and new isolates on a differential set of varieties

MUA for isolates

Naktuinbouw prepared the Standard agreement for the isolates (analogous to the MUA for the seed samples) and sent around for signing and collected the signed Standards agreements.

Candidate isolates

Regarding the isolates to be tested in the ring test, to limit the amount of work it was decided that all 10 new isolates would be tested by 4 participants and that the 7 standard reference isolates would be tested only by 3 participants. This meant that every participant had to test 4 new isolates and 2 old isolates (Naktuinbouw would test 3 old).

All isolates would be coded, but it was accepted to indicate in the coding if isolates concerned new isolates or old reference isolates. This was considered helpful information to be able for participants to organise the testing of the new Cf-9 breaking isolates with more strict isolation measures.
Naktuinbouw prepared a coding scheme according to the number of isolates to be tested for each participant.

Participants sent their chosen Cf-9 breaking isolates to Naktuinbouw before end of June, so Naktuinbouw would have 2 months for multiplication and preparation for sending before the deadline of beginning of September.
GEVES sent the race 0 and the race 2.4.5 from group E from the MATREF collection.
Naktuinbouw multiplied all the isolates and sent two plates of the 6 designated isolates according to the scheme and the 7 standard reference isolates to each participant beginning of September 2020.
Multiplication was tried on different media because of difference in growth between isolates. Finally however for each isolate one medium was chosen for multiplication for delivery to participants.

Figure 1: Plates of isolates 0, A, B, C, D and E (illustrating typical colour variations when multiplied on different media).

Differentials

Regarding the differentials for the ringtest, according to the project plan everybody would test with the 10 differentials (8 existing from MATREF and 2 candidates from Naktuinbouw). It was decided that everybody would receive the seeds of these 10 differentials under code. But in addition also the susceptible differential Monalbo and the Cf-9 resistant differential IVT1154 were not coded as controls in the ringtest. This meant a total of 12 samples to be tested with the 6 sent isolates.
As 12 plants had to be tested per differential, this meant that for 6 isolates to be tested, everybody needed at least 72 seeds per differential. Regarding germination etc. we decided to sent samples of 200 seeds of each differential to each participant.
With 10 participants a total stock of 2000 seeds was needed.

GEVES and Naktuinbouw checked if this amount of seeds would be available. GEVES could confirm, but Naktuinbouw found both seedlots were too small and ordered additional seeds from the same batches. These were sent by ENZA and Bayer.

Naktuinbouw prepared all the samples and sent to participants beginning of September 2020.

Test protocol

The resistance tests should be carried out according to the CPVO protocol of tomato (see the Annex). But different from the protocol, each isolate should be tested on at least 12 plants of each differential instead of 20 plants.
As regarding the notation scale and interpretation rule, it was decided that differently from the CPVO protocol, an IR reaction could be taken into account and that the following notation scale would be used:
1. (strong) sporulation (velvety, white spots)
2. yellowish spots without sporulation or with slight localised sporulation
3. no symptoms
As interpretation rule was decided that 1 = S, 2 = IR, 3 = R.
This was reflected in the final result sheet for delivering the results prepared by Naktuinbouw.

Sending results
All participating laboratories carried out the ring test and sent their results before or just after the deadline of end of March 2021.
Only lab 9 did not succeed in carrying out the ring test. Because of lack of time and space they only tried to start in March 2021, but then the isolates were not viable anymore.
A resending of isolates in September was unsuccessful, because the sending never reached lab 9 and was probably destroyed.
Unfortunately it had to be decided that although results would be missed, the project could not wait for further delay and isolates would not be sent again.

Marker results
It was mentioned in the project plan that presently marker tests had been developed to detect for certain Cf-genes of *Passalora fulva* in tomato varieties. Naktuinbouw had developed markers for the resistance genes Cf-4 (resistant to group A and D) and gene Cf-9 (resistant to races A to E) on chromosome 1.
It was asked that participating companies provide information about markers which are used by their company to screen for resistance to *Passalora fulva*.
Only Naktuinbouw had marker results available of some differentials used in the ring test.

Step 5. Development of a new set of tomato differentials carrying commonly and not commonly used Cf-genes.

Results of ring test

Naktuinbouw prepared the meeting of 30 September 2022 in which the results of the ring test were presented and discussed following a power point presentation.

Below, in table 7 and 8 the results of the ring test for the new Cf-9 breaking isolates and the old standard reference isolates are presented. In both tables also results of the initial characterisation (indicated as IC) and indication in the table on the ISF website (indicated as ISF) are given as a comparison with previous results and existing information.

The following observations could be made:

- One lab (9) is missing, no results will come available (isolates lost)
- Overall quite consistent results except for some S/IR and IR/R discrepancies, esp. in V121 (Cf-1) and Stirling Castle (Cf-3)
- Difference between S and IR sometimes not clear. This can also explain some discrepancies in the ringtest as for differentials V121 (Cf-3) and Stirling Castle (Cf-1) and for isolate N10
- Sometimes also difference between R and IR not clear, this is observed several times in the ringtest
- However IR is quite consistently found for some cases, like Purdue 135 (Cf-4) with isolate N9 and V121 (Cf-3) with isolate O14 (D)

- Stirling Castle (Cf-1) results are not consistent. For many differentials it is sometimes showing IR or even R for one lab compared to S for the other labs. Even doubts for old isolate 0 (yellow). Interpretation of IR could partly explain these.
- V121 (Cf-3) results are not consistent for some isolates. Some labs are judging it sometimes R compared to the rest (S). Even ISF information is doubtful for old isolate 0 and probably for A, B and E/2.4.5 (yellow). Interpretation of IR could partly explain these.
- Ontario 7818 (Cf-6) results are sometimes not consistent. Some labs are judging Ontario 7818 more S than the rest (R), even for old isolate B. Difficult to explain.

	code	(1),(3),4,5,9					(1),2, (3),9					(1),(2),3,(9)					1,3,4,9					1,2,3,9					1,2,(3),9					(1),2,3,9					1,2,3,((6)),(9)					(1),3,9					((1)), 2, 3, (6), 9, (C, C)				
	strain	N1					N2					N3					N4					N5					N6					N7					N8					N9					N10				
variety	lab	IC	1	8	9	10	IC	1	2	9	10	IC	1	2	3	10	IC	1	2	3	4	IC	2	3	4	5	IC	3	4	5	6	IC	4	5	6	7	IC	5	6	7	8	9	IC	6	7	8	9	IC	7	8	9	10
7 Monalbo	-	S	S	S		S	S	S	S		S	S	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	S	S		S	S	S	S		S	S	S		S
10 Stirling Castle	Cf-1	S	HG	S		IR	S	IR/R	S		S	-	-	S	HG	S	S	R	S	S	S	-	S	S	IR	S	S/IR	S	IR	S	S	S	IR	S	S	IR	-	S	-	S	IR	S	S	S	IR		S/IR	IR	S		IR
6 Vétomold	Cf-2	R	R	R		R	S	S	S		S	S	-	S	HG	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	S	HG		R	R	R	R		S	S	S		S
9 V121	Cf-3	S	S	IR		R	S	R	S		S	-	-	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S	S	S	-	S	S		S	S	S	HG		S	S	S		S
1 Purdue 135	Cf-4	S	S	S		S	R	R	IR		R	R	-	R	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	IR	R	R	R	IR	R	R	-	R	IR	IR	IR	IR	IR		R	R	R		R
2 IVT 1149	Cf-5	S	S	S		S	R	R	R		R	R	-	R	R	R	R	R	R	IR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	-	R	R	R	R	R	R		R	R	R		R
8 Ontario 7818	Cf-6	R	R	R		R	R	R	S		R	R	-	R	R	R	R	R	IR	HG	R	-	R	R	R	S	R	R	R	S	R	R	R	S/IR	IR	R	-	S	-	R	R	R	R	R		S	S	S		IR
3 IVT 1154	Cf-9	S	S	S		S	S	S	S		S	S	-	S	IR	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	S	S		S	S	S	S		S	S	S		S
4 Completo	Cf-9 +?	R	R	R		R	R	R	IR		R	-	-	R	IR	R	R	R	R	R	R	IR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	IR	R	-	R	R	R	R	R		S	S	S		IR
5 Chelino	Cf-9 +?	R	R	R		R	R	R	IR		R	-	-	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	IR	R	R	-	R	R	R	R	R		S	S	S		IR
Monalbo	-	S	S	S		S	S	S	S		S	S	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	S	S		S	S	S	S		S	S	S		S
IVT 1154	Cf-9	S	S	S		S	S	S	S		S	S	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	-	S	S		S	S	S	S		S	S	S		S

Table 7. Ringtest results of new Cf-9 breaking isolates per lab (IC = initial characterisation)

	code																																																	
	strain	O11 (0)					O13 (A)					O16 (B)					O12 (C)					O17 (D)					O14 (E)					O15 (2.4.5)																		
variety	lab	IC	ISF	1	7	8	IC	ISF	2	3	9	IC	ISF	5	6	10	IC	ISF	1	2	8	IC	ISF	6	7	10	IC	ISF	3	4	9	IC	ISF	4	5	10														
7 Monalbo	-	S	S	S	S	S	S	**S**	S	S		S	S	S	S	S	S	S	S	**S**	**S**	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S														
10 Stirling Castle	Cf-1	S	R	S	S	IR	R	**R**	IR	R		S	R	S	S	IR	S	S/R	S	**S**	**S**	R	R	S	IR	IR	IR		S	IR		S		IR	S	IR														
6 Vétomold	Cf-2	R	R	R	R	R	S	**S**	S	IR		R	R	R	R	R	S	S	S	**S**	**S**	R	R	R	R	R	S	S	S	S		S	S	S	S	S														
9 V121	Cf-3	S	R	S	S	S	IR/S	**R**	S	S		IR	R	S	S	S	R	R	R	**S**	**S**	IR	IR	IR	IR	S	IR	R	S	S		S	R	IR	S	S														
1 Purdue 135	Cf-4	S	R	R	R	IR	R	**R**	R	R		S	S	S	S	IR	S	S	S	**S**	**S**	R	R	R	R	R	S	S	S	S		S	S	S	S	IR														
2 IVT 1149	Cf-5	R	R	R	R	R	R	**R**	R	R		R	R	R	R	R	R	R	R	**R**	**IR**	S	S	S	S	S	S	S	S	S		S	S	S	S	S														
8 Ontario 7818	Cf-6	R	R	R	R	R	R	**R**	R	R		R	R	S	R	R	R	R	R	**R**	**R**	R	R	R	R	R	R	R	R	R		R	R	R	IR	R														
3 IVT 1154	Cf-9	R	R	R	R	R	R	**R**	R	R		R	R	R	R	R	R	R	R	**R**	**IR**	R	R	R	R	R	R	R	R	R		R	R	R	R	R														
4 Completo	Cf-9 +?	R		R	R	R	R		R	R		R		R	R	R	R		R	**R**	**IR**	R		R	R	R	R		R	R		R		R	R	R														
5 Chelino	Cf-9 +?	R		R	R	R	R		R	R		R		R	R	R	R		R	**R**	**IR**	R		R	R	R	R		R	R		R		R	R	R														
Monalbo	-	S	S	S	S	S	S	**S**	S	S		S	s	S	S	S	S	S	S	**S**	**S**	S	S	S	S	S	S	S	S	S		S	S	S	S	S														
IVT 1154	Cf-9	R	R	R	R	R	R	**R**	R	R		R	R	R	R	R	R	R	R	**R**	**IR**	R	R	R	R	R	R	R	R	R		R	R	R	R	R														

Table 8. Ringtest results of old standard reference isolates per lab (IC = initial characterisation, ISF indication on ISF website)

		isolate	N9	N2	N3	N5	N6	N7	N8	N4	N1	N10
		code	Ff-80	G18.035	DE1225	TS-36	U146	Ff-72	TS-38	Ff-77	Ff-86	AB 187
		company	Sakata JP	Gautier	RZ	Bayer	Enza	Sakata JP	Bayer	Sakata JP	Sakata JP	Enza
		origin	Japan	France dpt 44 w	Germany	UK	Netherlands NB	Japan	France	Japan	Japan	South-Korea
Monalbo	-		S	S	S	S	S	S	S	S	S	S
Stirling Castle	Cf-1		S	S	S	S	S	S/IR	S	S	S	IR
Vétomold	Cf-2		R	S	S	S	S	S	S	R	R	S
V121	Cf-3		S	S	S	S	S/R	S	S	S	S/IR/R	S
Purdue 135	Cf-4		IR	R	R	R	R	R/IR	R	S	S	R
IVT 1149	Cf-5		R	R	R	R	R	R	R	R	S	R
Ontario 7818	Cf-6		R	R	R	R	R	R/IR	R/S	R	R	S
IVT 1154	Cf-9		S	S	S	S	S	S	S	S	S	S
Completo	Cf-9 + (Cf-6 or new?)		R	R	R	R	R	R	R	R	R	S
Chelino	Cf-9 + (Cf-6 or new?)		R	R	R	R	R	R	R	R	R	S
			(1),3,9	(1),2, (3),9	(1),(2),3,(9)	1,2,3,9	1,2,(3),9	(1),2,3,9	1,2,3,((6)),(9)	1,3,4,9	(1),(3),4,5,9	)), 2, 3, (6), 9, (C, C)
		proposed	x			x				x	x	x
						most consistent results						

Table 9. Ringtest results of new Cf-9 breaking isolates summary per isolate

The following conclusions about the old standard reference isolates could be drawn:

- Results for old isolates mostly consistent and mostly according to expectations (ISF table)
- Results for the commonly used genes Cf-2, Cf-4, Cf-5 and Cf-9 are all consistent and as expected
- Unexpected results for Cf-1 and Cf-3 with old isolates 0 and B (yellow). It is doubtful if race 0 is really the right isolate. Is this due to interpretation IR or is ISF information not correct for Cf-1 and Cf-3?
- If isolate 0 is the right one, the “theory” that any resistance gene gives resistance to isolate 0 is not correct. Should we rename or skip?
- Old strains E and 2.4.5 show similar results in the ringtest according to expectations ISF table. Differences in the initial characterization for Cf-3 are not confirmed in the ringtest and could be caused by interpretation of IR.
- The isolate of group C in the ringtest concerns 1,2,4 (see Table 1 (1,2,4, or 2,4))

Looking in table 9 with a summary per isolate of Cf-9 breaking isolates, the following conclusions about the new Cf-9 breaking isolates could be drawn:

- At least 5 distinct new Cf-9 breaking isolates (pathotypes). 4 unique isolates N1, N4, N9 and N10 and a group with the 6 isolates N2, N3, N5, N6, N7 and N8
- All of them break Cf-9 (and Cf-1 and Cf-3), but except N9 also different combinations of Cf-2, Cf-4 and Cf-5.
- The group of 1,2,3,9 isolates (N2, N3, N5, N6, N7 and N8) shows only slight differences which could be caused by differences in interpretation only
- N5 is in that group the isolate with most consistent results
- The geographical origin of isolates in the group is Europa except for N7
- The geographical origin of all unique isolates is Asia
- The isolates which were unique in the initial characterization are also unique in the ringtest, except for N6 which seems now quite similar to the rest of the group. This possibility was already indicated at time of initial characterization.
- N10 shows very clear and distinct results with even breaking genetics in Completo and Chelino and Cf-6
- This means that the conclusion from Iida in 2015 that the Cf-6 pattern is still not broken is not true anymore (interestingly, N10 was already isolated in S-Korea in 2014)

The following conclusions about the new differentials could be drawn:

- The varieties with new genetics (Completo and Chelino) show in this ringtest the same and very consistent results. These new genetics could be the same.
- Chelino and Completo show the same resistance pattern as the known gene Cf-6 in Ontario 7818.
- According to marker tests of Naktuinbouw Chelino and Completo contain also Cf-9 and hence are not monogenic.

Discussion on results of ring test.

During the meeting and especially at Questions/discussion the following points were discussed, choices made between several options and decisions taken to resolve all necessary issues to finish the project and make the report and publications.

1. Do we need additional tests to explain/confirm doubtful results?

It seems that a lot of the discrepancies in results in the ringtest were caused by the fact that interpretation of IR was not so clear.
Most of these discrepancies were in the combination with Cf-1 and Cf-3 differentials.
It was mentioned that both genes are based on a slower mechanism, that shows resistance in a later stage than most other genes. This could have been one of the reasons for the discrepancies. Particularly the Cf-9 gene causes hypersensitivity that can lead to different phenotypes than only sporulation (pinpoints, (brown necrotic) spots, flacks, plant death) which could be confused with the IR reaction with especially Cf-1 and Cf-3.
However it was concluded that all mechanisms lead to a resistance level which is not really intermediate but at a high level.

It was agreed that the differences in the group of six 1,2,3,9 isolates could be caused by differences in interpretation only. The proposal to denominate only isolate N5, with the most stable and consistent results from that group, was accepted.

2. Use of IR, notation scale and interpretation rule

In this respect it was discussed and proposed that for the notation scale it is good to keep the three different classes, but to improve the notation scale for the biotest a little regarding sporulation and brown spots as follows. Besides to rearrange the order with the lowest note for the class with the least symptoms and the highest note for the class with most symptoms as common practice in other protocols.

1 = no symptoms or brown necrotic spots without sporulation (R)
2 = yellowish spots without sporulation and/or slight localized velvety white spots (R)
3 = velvety, white or brown spots with sporulation

For the test protocol for UPOV and CPVO, it was agreed to amend the notation scale accordingly.

Regarding the results in the differential table it was agreed that because as mentioned the different resistance mechanisms all lead to a HR resistance level, the IR level can be avoided from the conclusion and deleted from the differential table.
It was proposed however to explain in the ISF Pf differential host document the fact that two mechanisms exist which lead to two symptom classes but which both are interpreted as HR.

For the test protocol for UPOV and CPVO it was agreed that for some races we will add standard varieties with notation scale 2 for more clarity. As can be seen in tables 7 and 8 it concerns Purdue 135 (Cf-4) with isolate N9 and V121 (Cf-3) with isolate O14 (D). These had been tested 4 resp. 3 times in this project (including the initial characterisation) with the same consistent IR result.

3. Is race 0 the right isolate or has it changed? Need to rename or skip?

We discussed we do not know if race 0 has changed. It is mentioned that several other isolates called race 0 showed the same results. We decided we can still call it race 0. When we skip the differentials for Cf-1 and Cf-3, it still shows resistance for all differentials in our table except the one without resistance genes.
The initial characterization suggested that it should break Cf-4, however this was not confirmed in the ringtest.

4. Do we need the not commonly/commercially used Cf-genes (Cf-1, Cf-3) in the new differential set or can we skip these?

The need for the Cf-1 and Cf-3 differentials was discussed quite intensively and it was agreed that as these are not needed to discriminate the 11 different isolates, we can skip the two differentials from the differential table.
Our main goal is being practical and in relation to the marketing of varieties, but we always also want to be in line with scientific knowledge.
But with many inconclusive and inconsistent results for these two differentials, including them would in this case cause more confusion than clarity. So it is agreed to skip both from the table.

5. Are we sure that Ontario 7818 only contains Cf-6? (markertest for Cf-9?)
6. Do Chelino/Completo contain existing Cf-gene Cf-6 or another gene?

Companies did not want to disclose the genetic background of the new differentials Completo and Chelino.
But as results for both differentials anyway do not differ from the Cf-6 differential, it was agreed that an answer on both questions is in fact not essential and we do not need to include Completo and/or Chelino as new differentials for the moment.

7. How do we denominate the (new) isolates (letter (F to J?), Pf:code) or sextet code)?

The options are presented in table 10 below.

		isolate	0	A	B	C	D	E	N9	N5	N4	N1	N10
		code							Ff-80	TS-36	Ff-77	Ff-86	AB 187
		company							Sakata JP	Bayer	Sakata JP	Sakata JP	Enza
		origin							Japan	UK	Japan	Japan	South-Korea
Monalbo	-		S	S	S	S	S	S	S	S	S	S	S
Stirling Castle	Cf-1		S/IR/R	R	S/IR/R	S	R/IR	IR	S	S	S	S	IR
Vétomold	Cf-2		R	S	R	S	R	S	R	S	R	R	S
V121	Cf-3		S?	S/IR/R	S?	S/R	IR	S/IR	S	S	S	S/IR/R	S
Purdue 135	Cf-4		R	R	S	S	R	S	IR	R	S	S	R
IVT 1149	Cf-5		R	R	R	R	S	S	R	R	R	S	R
Ontario 7818	Cf-6		R	R	R	R	R	R	R	R	R	R	S
IVT 1154	Cf-9		R	R	R	R	R	R	S	S	S	S	S
Completo	Cf-9 + (Cf-6 or new?)		R	R	R	R	R	R	R	R	R	R	S
Chelino	Cf-9 + (Cf-6 or new?)		R	R	R	R	R	R	R	R	R	R	S
		old	1,3?	2,3?	3,4?	1,2,4	5	2,4,5	(1),3,9	1,2,3,9	1,3,4,9	(1),(3),4,5,9	((1)), 2, 3, (6), 9, (C, C)
		new (without Cf-1, Cf-3)	0	2	4	2,4	5	2,4,5	9	2,9	4,9	4,5,9	2,6,9
		letter	0	A	B	C	D	E	F	G	H	I	J
		Cf:code	Cf:0?	Cf:1	Cf:2	Cf:3	Cf:4	Cf:5	Cf:6	Cf:7	Cf:8	Cf:9	Cf:10

Table 10. Possibilities for a new differential table of *Passalora fulva* in tomato

The first two options (using a letter from A to J or Pf:code with a number) were discussed quite intensively.
Regarding the choice between a letter or a Pf:code with a number, it was argued that although the normal practice is to use numbers, users are very much used to letters and the system with letters is much easier to explain. Changing to the Pf:code with numbers would in this respect ask much more explanation and could cause difficulties and confusion.
Therefore it was agreed to use the letter system.
Regarding the naming of the new races, it was agreed to use figure 0 and letters A to J, with F to J for the new races.

It was also proposed and accepted to swap N9 and N5 with the letters F and G, as in this case races A to F would concern races appearing mostly in Europe and races G to J races appearing mostly in Asia. This reflects also the experience that there is limited variability in Cf-9 breaking races across Europe (only isolate N5 (to be called F)).
The option to use both letters and Pf-code with a number was not favoured, since it could also lead to more confusion than clarity.

There was not much discussion about the option of adding also a sextet code. It was mentioned that at the moment we do not need it, because unlike as with Bremia in lettuce and Peronospora in spinach, we do not have a yearly evaluation of hundreds of new races which need to be evaluated for addition in the system.

Step 6. Definition of a new coding system

Following the conclusions of the discussion in the meeting of 30 September 2021, the following differential table based on the denomination rules of ISF is proposed.
An explanation should be added that the letters correspond to isolates and not to a group of isolated anymore as in the old situation.
As mentioned above, it should also be explained that two resistance mechanisms exist which lead to two symptom classes but which both are interpreted as HR.

Passalora fulva (Pf) - **Tomato**

		Pf:	0*	A*	B*	C*	D*	E*	F*	G*	H*	I*	J*
		virulence	0	2	4	2,4	5	2,4,5	2,9	9	4,9	4,5,9	2,6,9
Differential hosts	**Cf-gene**												
Monalbo*	-		S	S	S	S	S	S	S	S	S	S	S
Vétomold*	Cf-2		HR	S	HR	S	HR	S	S	HR	HR	HR	S
Purdue 135*	Cf-4		HR	HR	S	S	HR	S	HR	HR	S	S	HR
IVT 1149*	Cf-5		HR	HR	HR	HR	S	S	HR	HR	HR	S	HR
Ontario 7818*	Cf-6		HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	S
IVT 1154*	Cf-9		HR	HR	HR	HR	HR	HR	S	S	S	S	S

S = susceptible
HR = highly resistant
*differential hosts and isolates that are used by the seed sector

Table 11. Proposal for new differential host table ISF

After the project the new strain(s) and new differential(s) will be stored at Naktuinbouw,(Naktuinbouw – Plantum isolate collection, only isolates) and MATREF (isolates and differentials) as for the old isolates and differentials.

Step 7. Publication of the results

When the project is finished, the results will be published in 2022.
The new coding system for *Passalora fulva* will be published by ISF on its website at the differential hosts.
In the meeting of 30 September 2022, it was proposed that in addition we prepare a poster (as for TEV in pepper) for the next Eucarpia tomato meeting (Valencia, May 31 – June 3 2022).
It was also proposed to prepare an article for the KNPV (Plant protection) magazine in the Netherlands as was done in 2019 for the ISF DRT procedures and principles.
Also publication in some growers magazines like the Dutch Groente en Fruit was suggested.

Preparation of, participation in and coordination of the project
Naktuinbouw coordinated this project.

Participating companies are listed in the table 12 below.

Participants:

Company	Contact name	email
Naktuinbouw	Wim Sangster Diederik Smilde	w.sangster@naktuinbouw.nl d.smilde@naktuinbouw.nl
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Table 12. Participants of the ISF *Passalora fulva* project

Meetings of the project:

Kick-off meeting online on 3 December 2019
Meeting on results initial characterisation online on 22 April 2020
Meeting on ring test results on 30 September 2021

References:

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Annex

Ad. 47.1 - 47.7: Resistance to *Fulvia fulva* (Ff) (ex *Cladosporium fulvum*)

1. Pathogen	*Fulvia fulva* (ex *Cladosporium fulvum*)
3. Host species	*Solanum lycopersicum*
4. Source of inoculum	Naktuinbouw[10] (NL) or GEVES[11] (FR)
5. Isolate	Race group 0, A, B, C, D, and E
6. Establishment isolate identity	with genetically defined differentials from GEVES (FR) A breaks Cf-2, B Cf-4, C Cf-2&4, D Cf-5, E Cf-2&4&5
7. Establishment pathogenicity	symptoms on susceptible tomato
8. Multiplication inoculum	
8.1 Multiplication medium	Potato Dextrose Agar or Malt Agar or a synthetic medium
8.8 Shelf life/viability inoculum	4 hours, keep cool
9. Format of the test	
9.1 Number of plants per genotype	more than 20
9.2 Number of replicates	Not applicable
9.3 Control varieties	
Susceptible:	Monalbo, Moneymaker
Resistant for race 0:	Angela, Estrella, Sonatine, Sonato, Vemone, Vagabond, IVT 1149, Vagabond × IVT 1149, IVT 1154
Resistant for race group A:	Angela, Estrella, Sonatine, Sonato
Resistant for race group B:	Angela, Estrella, Sonatine, Sonato, Vemone
Resistant for race group C:	Angela, Estrella, Sonatine
Resistant for race group D:	Estrella, Sonatine, Vemone
Resistant for race group E:	Sonatine, Jadviga, Rhianna, IVT 1154
9.5 Test facility	glasshouse or climate room
9.6 Temperature	day: 22° C, night: 20°or day: 25°C, night 20°C
9.7 Light	12 hours or longer
9.9 Special measures	depending on facility and weather, there may be a need to raise the humidity, e.g. humidity tent closed 3-4 days after inoculation and after this, 66% until 80% closed during day, until end
10. Inoculation	
10.1 Preparation inoculum	prepare evenly colonized plates, e.g. 1 for 36 plants; remove spores from plate by scraping with water with Tween20; filter through double muslin cloth
10.2 Quantification inoculum	count spores; adjust to 10^5 spores per ml or more
10.3 Plant stage at inoculation	19-20 d (incl. 12 d at 24°), 2-3 leaves
10.4 Inoculation method	spray on dry leaves
10.7 Final observations	14 days after inoculation
11. Observations	
11.1 Method	visual inspection of abaxial side of inoculated leaves
11.2 Observation scale	Symptom: velvety, white spots
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
11.4 Off-types	excessively high humidity may cause rugged brown spots on all leaves

12. Interpretation of data in terms of UPOV characteristic states

absent	[1]	symptoms
present	[9]	no symptoms

13. Critical control points:

Ff spores have a variable size and morphology. Small spores are also viable.
Fungal plates will gradually become sterile after 6-10 weeks. Store good culture at -80°C.
For practical purposes, it is not possible to keep plants longer than 14 days inside a tent.

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