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THE REACTION OF *CHRYSANTHEMUM* CULTIVARS TO *PUCCINIA HORIANA* AND THE INHERITANCE OF RESISTANCE

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SUMMARY

Four types of reaction of *Chrysanthemum morifolium* to infection by *Puccinia horiana* are described. (1) Resistant plants show no macroscopic lesions. Cultivars classified as resistant carry a single dominant gene mostly in a simplex, sometimes in a duplex condition. (2). Incomplete resistance provides good protection but under extreme test conditions some pustules develop slowly. (3) Necrosis inhibits spore formation to a large extent. The progenies of crosses between necrotic and susceptible cultivars are mostly susceptible. (4) Susceptible plants sporulate abundantly.

The merits of the three types of resistance are discussed.

INTRODUCTION

Puccinia horiana HENN. is a parasite of *Chrysanthemum morifolium* RAMAT. that causes white rust. It is a microcyclic rust producing teleutospores which germinate in situ and the basidiospores reinfect the chrysanthemum. Resistance to the fungus is available and from 1975 onwards resistant cultivars are being bred in the Netherlands. No breakdown of resistance has been reported in these cultivars, although this was expected in view of the evidence of physiological specialisation (MARTIN & FIRMAN, 1970; DICKENS, 1971; YAMAGUCHI, 1981). To cope with a possible breakdown, the resistances available were studied to draw parallels with the vast body of information available on rust in cereals. This paper reports on the various types of reaction of Chrysanthemum to infection with *Puccinia horiana* and on the genetic basis of these reactions.

MATERIALS AND METHODS

Host genotypes. Based on pustule development the following four types of interaction between *C. morifolium* and *P. horiana* were defined:

1. No symptoms visible, no spore production (complete resistance, R).
2. Few pustules that develop slowly and sporulate limitedly (incomplete resistance, IR).

3. Necrotic areas develop around the growing rust colonies, sporulation may not be completely inhibited (necrosis, N).
4. Many rust pustules that develop quickly and sporulate abundantly (susceptible, S).

Crosses were made between cultivars representing these four reaction types.

Pathogen genotype. The Dutch Plant Protection Service (PD) supplied the pathogen which was collected from several nurseries in the Netherlands, mixed and subsequently maintained on young cuttings of susceptible cultivars.

Inoculation method. Sporulating plants were placed for 24 h among seedlings or parent clones in a transparent polythene covered greenhouse bench. In the polythene tunnel a film of free water settled on the plants, a pre-requisite for successful germination of basidiospores (FIRMAN & MARTIN, 1968). The temperature was maintained at 16 to 18 °C. Seedlings were tested in the three to four true leaf stage, while for the parent clones rooted cuttings with about eight leaves were used. Tests had shown earlier that this difference in development does not affect the interaction type. About ten days after inoculation symptoms appeared. On days 14, 21 and 28 the reaction types were scored and condensed into three classes. All plants with pustules (reaction types S and IR) were classified susceptible S. The term necrosis, N, was used for plants that showed necrotic areas and resistance, R, for plants without symptoms.

The number of plants tested is shown in Tables 3–7. Five experiments were run and in each experiment the plants were split into two groups that served as replications. Experiments were linked through the F_1's and parents they had in common. Resistant plants were flowered to verify their parentage through flower color and -shape.

Segregation. C. morifolium is an outbreeding vegetatively propagated crop which segregates in the F_1 generation. It is a complex hybrid (DOWRICK, 1953) with $2n = 6\times = 54$ chromosomes in which, at meiosis, bivalent formation is the norm and multivalents are rare (WATANABE, 1977). The bivalents may be formed in two ways: the two most homologous chromosomes may pair up (preferential pairing) or the six chromosomes may pair at random to form three bivalents. The expected segregation ratios for monogenic inheritance (Table 1) depend on which system operates (LANGTON, 1980). Deviations from the expected ratios may occur in aneuploids which are frequently found in *C. morifolium* (DOWRICK, 1953; ENDO, 1969). χ^2's were computed to test the goodness of fit.

RESULTS

No significant differences between experiments could be shown and data were combined over all experiments.

Table 2 shows the response of the parent cultivars used. The susceptible cultivars produced pustules easily and quickly although there was some variation e.g. 'Delta' formed large pustules, while IVT 80061-3 responded with very large numbers of small pustules, which also appeared a little later.

Resistant cultivars did not show any visible symptoms throughout the experiment.

Table 1. Expected segregation ratios for a one locus single dominant gene coding for resistance in hexaploid Chrysanthemum. The two models assume the formation of bivalents at meiosis with either selective or random pairing.

Cross	Ratios expected in progeny (R:S)	
	selective pairing (diploid like inheritance)	random pairing (hexaploid inheritance)
Nulliplex × nulliplex	0:1	0:1
Simplex × nulliplex	1:1	1:1
Simplex × simplex	3:1	3:1
Duplex × nulliplex	3:1*	4:1
Duplex × simplex	7:1*	9:1
Duplex × duplex	15:1*	24:1

* With non-pairing dominant alleles, otherwise all progeny would be resistant.

Table 2. Reaction of parental clones to *Puccina horiana*. S = susceptible, R = resistant, IR = incompletely resistant, N = necrosis.

Parental clone	Origin	Number of cuttings tested	Cumulative number of sporulating cuttings on day			Type
			14	21	28	
IVT 79024-2	Holland	14	14			S
IVT 80058-1	Holland	10	10			S
IVT 80061-3	Holland	13	7	13		S
IVT 80109-3	Holland					S
IVT 80184-3	Holland					S
IVT 80189-4	Holland					S
Carella	Holland					S
Delta	Holland	17	17			S
Dark Pink Gin	England	24	23	24		S
Robeam	England	14	13	14		S
80211	Germany	13	0	0	0	R
Bridesmaid	Holland	17	0	0	0	R
Carillon	Holland					R
Dollaroid	Holland					R
Guilderland	Holland	24	0	0	0	R
Lameet	Holland	23	0	0	0	R
Renine	Holland	20	0	0	0	R
Rewilo	Holland					R
Roulette	Holland	77	0	0	0	R
Ready	Japan	32	0	0	0	R
Smile	USA	53	0	0	0	R
Refour	Japan	20	0	6	10	IR
Redemine	Holland	49	49*			N
Refla	Holland	10	10*			N

* Necrosis visible.

Table 3. F_1 seedling segregation for reaction to *Puccinia horiana* in crosses between resistant cultivars and the susceptible IVT 80058-1.

Resistant parent	Number of seedlings			Test ratio selective pairing		Test ratio random pairing	
	R	S	N	R:S	P	R:S	P
Bridesmaid	161	145	1	1:1	0.50		
Carillon	157	159	27	1:1	0.98		
Dollaroid	108	98	0	1:1	0.50		
Guilderland	253	249	1	1:1	0.90		
Ready	94	63	0	1:1	0.02		
Rewilo	41	57	16	1:1	0.20		
Roulette	113	114	0	1:1	0.98		
Smile	146	128	0	1:1	0.30		
80211	112	27	0	3:1	0.20	4:1	0.90

On 'Refour' symptoms developed slowly and sparsely. In commercial greenhouses this cultivar seldom shows any rust pustules, but under the extreme conditions of the test (free water on leaves, high infection pressure) a few small pustules were formed, that were able to sporulate.

The cultivars Refla and Redemine showed necrotic lesions upon infection. The lesions were three mm in diameter and close inspection of a few leaves learned that the formation of teleutospores was not completely prevented. Those of 'Refla' also produced basidiospores. The reaction of parents of which no data are shown was determined in earlier tests.

Segregations. Five hundred and eighty F_1-seedlings of four crosses between susceptible cultivars were assessed. They were all susceptible except one seedling which was resistant. This resistant seedling of the cross 'Dark Pink Gin' × IVT 80061-3 could be due to an illegitimate outcross.

Crosses between resistant cultivars and the susceptible IVT 80058-1 segregated in

Table 4. F_1 seedling segregation for reaction to *Puccinia horiana* in crosses between resistant cultivars and the susceptible 'Delta'.

Resistant parent	Number of seedlings			Test ratio selective pairing		Test ratio random pairing	
	R	S	N	R:S	P	R:S	P
Bridesmaid	131	181	2	1:1	0.01		
Carillon	207	261	3	1:1	0.02		
Dollaroid	86	107	0	1:1	0.20		
Roulette	206	232	0	1:1	0.30		
Smile	100	108	0	1:1	0.70		
Ready	117	64	1	1:1	0.001		
80211	162	66	0	3:1	0.20	4:1	0.001

Table 5. F_1 seedling segregation for reaction to *Puccinia horiana* in crosses between resistant cultivars.

Cross		Number of seedlings			Test ratio selective pairing		Test ratio random pairing	
		R	S	N	R:S	P	R:S	P
Carillon	× Rewilo	99	48	0	3:1	0.05	4:1	0.001
Dollaroid	× Rewilo	55	17	8	3:1	0.80	4:1	0.50
Guilderland	× Rewilo	102	47	0	3:1	0.10	4:1	0.001
Lameet	× Rewilo	125	43	0	3:1	0.90	4:1	0.10
Bridesmaid	× 80211	145	23	0	7:1	0.70	9:1	0.20
Carillon	× 80211	274	41	1	7:1	0.80	9:1	0.10
Dollaroid	× 80211	186	24	0	7:1	0.70	9:1	0.50
Guilderland	× 80211	196	37	0	7:1	0.20	9:1	0.01
Rewilo	× 80211	170	23	0	7:1	0.90	9:1	0.50
Roulette	× 80211	191	24	0	7:1	0.70	9:1	0.70
80211	× Renine	240	18	0	15:1	0.70	24:1	0.02

a 1:1 ratio for resistance (R) and susceptiblity (S), suggesting that a single dominant gene controls resistance (Table 3). The 3:1 segregation of the cross 80211 × IVT 80058-1 indicated that 80211 carries two dominant alleles of the gene coding for resistance and is therefore a duplex.

In the progeny of the crosses 'Carillon' and 'Rewilo' a fair number of necrotic types was found indicating that another factor affecting the interaction between host and pathogen was involved. Plants with necrosis were neither R nor S and were excluded from the χ^2 test. 'Ready' had a higher number of resistant plants in the progeny than expected and it will be shown later that this cultivar combines a major gene for resistance with genes for incomplete resistance.

In crosses between resistant cultivars and the susceptible 'Delta', the progenies of 'Bridesmaid', 'Carillon' and 'Ready' deviated from the 1:1 ratio (Table 4). The cross 'Bridesmaid' × 'Delta' showed a normal development of the pathogen in the seedlings. In contrast to the cross with IVT 80058-1, hardly any necrosis was now found in the progeny of 'Carillon'. In the 'Ready' cross, seedlings with a long latent period were found suggesting the presence of genes for incomplete resistance.

Comparison of Tables 3 and 4 showed that when IVT 80058-1 and 'Delta' are crossed with the same resistant cultivar, the progenies of IVT 80058-1 hold more resistant plants than those of 'Delta'.

A number of resistant cultivars was crossed with the resistant 'Rewilo' (simplex) and 80211 (duplex). Table 5 shows that in the two 'Rewilo' progenies that deviated from the 3:1 ratio fewer resistant seedlings occurred than expected. All crosses with 80211 segregated as expected. Another proposed duplex is the cultivar Renine. In the crosses with 80211 the selective pairing model fits better than the random type one.

Incomplete resistance. 'Refour' was identified as incompletely resistant because of the long latent period and small pustules. Most seedlings from crosses between 'Refour' and susceptible cultivars developed sporulating lesions within 14 days, which is the

Table 6. F_1 seedling segregation for reaction to *Puccinia horiana* in crosses between cultivars with incomplete resistance and susceptible cultivars.

Cross	Number of seedlings			Number of susceptible seedlings at ... days after inoculation			
	R	S	N	14	21	28	35
Refour × IVT 80058-1	70	182	0				
Refour × Delta	25	182	0				
Refour × IVT 80184-3	26	156	0	107	148	155	156
Refour × IVT 80089-4	22	197	2	150	181	186	197
Refour × 80211	72	15	2	11	15	15	
Ready × Delta	117	64	1	21	54	59	64
Ready × IVT 80184-3	121	73	0	32	53	68	73
Ready × IVT 80058-1	94	63	0				

Table 7. F_1 seedling segregation for reaction to *Puccinia horiana* in crosses between cultivars showing necrosis and susceptible cultivars.

Cross	Number of seedlings		
	R	S	N
Refla × IVT 80061-3	9	173	1
Redemine × IVT 80058-1	10	165	0
Redemine × Delta	0	197	2

common susceptible reaction. A smaller number of seedlings showed delayed development of pustules and there was also a group that remained free of symptoms (Table 6). The segregation ratios, which are not 1: 1 further suggest that 'Refour' does not carry a monogenic resistance factor.

In contrast with 'Refour', the cultivar Ready did not show any pustules and was therefore thought to carry a single gene for resistance. The delayed pustule formation in the progeny of 'Ready' points to the presence of incomplete resistance also. The combined presence of a monogenic resistance gene and genes for incomplete resistance should give more than 50%resistant seedlings which explains the deviation from the 1:1 ratio reported in the 'Ready' progenies.

Necrosis. Crosses between cultivars that show necrosis and susceptible cultivars produced F_1 seedlings of which the majority was susceptible, with a small number of resistant plants (Table 7). Necrosis occurred only sporadically in the F_1: three among 647 F_1 seedlings. This frequency is of the same order as often found in other crosses involving parents with monogenic resistance. Again the cross with IVT 80058-1 yielded more resistant seedlings than the comparable cross with 'Delta'.

DISCUSSION

The overall results provide good evidence that a single dominant gene conditions resistance. We provisionally propose the symbol Ph for this gene. Deviations from the expected ratios could be explained by the occurrence of other mechanisms combined with monogenic resistance, such as incomplete resistance and necrosis. The consistently lower than expected number of resistant progeny in crosses with the susceptible 'Delta' also suggests that other factors than Ph alone are involved in the growth of the pathogen on the plant.

The preferential pairing hypothesis assumes that the chromosomes carrying the rust resistance gene do not pair, otherwise the progeny would be completely resistant. This assumption may not hold. The chromosomes that pair preferentially will be structurally related and the chance that related chromosomes both carry the Ph gene is larger than for not related ones. WATANABE (1983) concluded from a study with 4x colchiploids that bivalent formation is due to a pairing regulation system and not to homology of the chromosomes. The chromosomes would then pair at random giving hexasomic inheritance.

The segregations for resistance, however, often fit the preferential pairing hypothesis, giving diploid ratios, rather than the random pairing one, associated with hexaploid inheritance, though this is not absolutely convincing. Possibly, a combination of the two pairing types may be a reality.

Resistance based on hypersensitivity is common in many plant-rust combinations and is usually controlled by a single dominant gene (RUSSELL, 1978). The hypersensitive reaction may occur shortly after the formation of the first haustoria (early hypersensitivity) or at later times (late hypersensitivity); the reproduction may not even be prevented (NIKS & KUIPER, 1982). The necrosis reported in this paper seems comparable to late hypersensitivity. In the three instances where the necrotic lesions were examined sporulation was not completely inhibited. The outstanding difference with late hypersensitivity in cereals is the genetic basis which does not seem monogenic. This is best seen in the cross Necrosed × Susceptible, where the number of necrotic seedlings falls far short of the 1:1 ratio expected for monogenic inheritance. YAMAGUCHI (1981) who made similar crosses in *Chrysanthemum* found many more seedlings with necrosis although the variation between crosses was wide: 7–70%. Possibly late hypersensitivity results from a delicate balance between host and pathogen that is easily tipped by environmental factors or genetic background of which ESKES (1983) gives examples.

With early hypersensitivity the reaction is swift. Fungal growth is arrested without provoking macroscopically visible symptoms. Microscopy is needed to verify whether the monogenic resistance to *P. horiana* reported here is based on early hypersensitivity.

This and YAMAGUCHI's study (1981) have shown that considerable variation in resistance exists. Easiest to use is the Ph gene. So far, this gene has given a very effective control of *Puccinia horiana* but it could break-down, analogous to the break-down of resistance with type 0 reaction (no symptoms) in *Puccinia graminis* (VAN DER PLANK, 1968). Since 1975, when the Plant Protection Service started testing (CEVAT, personal communication) no break-down of resistance has been documented in Holland, suggesting that physiological specialisation plays no role as yet here.

Incomplete resistance to *P. horiana* has features in common with partial resistance, which is a form of incomplete resistance in which the spore production is reduced even though the host plants are susceptible to infection (PARLEVLIET, 1979). It is considered by many (ESKES, 1983) to be more durable than the resistances with a hypersensitive reaction type. The polygenic background of partial resistance makes it more difficult however to work with.

The advantage of complete resistance whether based on early hypersensitivity or not is the absence of the pathogen under all circumstances, which is not so with necrosis or incomplete resistance. Where zero tolerance for *Puccinia horiana* is enforced at international borders, the risk of the occasional spot cannot be taken. Under such circumstances plants with incomplete resistance should be monitored carefully and fungicides may still be needed for absolute control.

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