Journal of Nematology 25(2):265–269. 1993.

Partial Resistance to *Heterodera avenae* in Wheat Lines with the 6M^v Chromosome from *Aegilops ventricosa*[1]

R. Rivoal,[2] J. Jahier,[3] and M. Hulle[2]

Abstract: Lines of wheat with the 6M^v chromosome from *Aegilops ventricosa* display partial resistance to both pathotypes Ha12 and Ha41 of *Heterodera avenae*. With either pathotype, the effect of this alien chromosome on cyst production, size, and fecundity was expressed in resistance tests. Partial resistance of five 6M^v(6D) substitution lines varied according to the intrinsic cyst-forming capacity of the nematode pathotypes and the recipient germplasms. Such partial resistance can be utilized in wheat breeding lines for integrated management of the cereal cyst nematode.

Key words: addition line, *Aegilops ventricosa,* breeding, cereal cyst nematode, gene, *Heterodera avenae,* nematode, partial resistance, pathotype, resistance, resistance test, substitution line, *Triticum aestivum.*

Studies on the resistance of wheat to the cereal cyst nematode (CCN, *Heterodera avenae* Wollenweber) have revealed few genes for resistance in *Triticum aestivum* L. (5). The sources of monogenic resistance commonly used are lines Loros or Aus.10894 (3,14,15). To prevent the resistance breaking that might result from frequent use of this gene, the search for other resistance sources has been focussed on wild cereal species. A large variation in resistant reactions occurred in the subtribe Triticinae, which includes two species totally resistant to *H. avenae: Aegilops ventricosa* Tausch and *A. variabilis* Eig (6). The resistance of *A. ventricosa* was incompletely transferred to a partially resistant wheat addition line (line m36) with the added chromosome 6M^v (20).

Four French pathotypes of *H. avenae* (Ha11, Ha12, Fr2, and Ha41) differ in virulence on oat (cv. Peniarth and cv. Noire de Moyencourt), on barley (resistance genes Ha1 and Ha2 in cv. Drost and cv. Siri, respectively), but not on cv. Loros and Aus.10894 wheat (1,18). Northern Ha12 and southern Ha41 pathotypes were shown to be distinct, with specific temperature requirements for breaking diapause before juvenile emergence (19). The Ha12 and Ha41 pathotypes are also distinguished by their intrinsic capacity for female development on the same host genotype: 24 or 48 juveniles (J2) of Ha41 yielded two or three times more cysts than Ha12 on wheat cv. Hardi and barley cv. Aramir, respectively, grown in Petri dishes (22).

Although characterization of total or almost complete incompatibility of plants to nematodes is relatively easy by qualitative estimations (4), assessment of intermediate resistance is more difficult, requiring accurate tests comparing it with nematode multiplication on control hosts. To minimize variability in quantitative results obtained with potato cyst nematodes, a Pf/Pi ratio (final population density/initial population density) was calculated in terms of fecundity (eggs produced rather than cysts) (9). Substantial information is available for such resistance tests for *Globodera pallida* (Stone) populations (13,16,17).

In contrast, quantitative estimations of partial resistance in wheat to *H. avenae* have been undertaken rarely (5). Our objectives were i) to characterize the effect in wheat of the 6M^v chromosome of *A. ventricosa* on cyst production, size, and fecundity in relation to initial densities of pathotypes Ha12 and Ha41; and ii) to assess the level of resistance in 6M^v(6D) substitution lines. Experiments were made with both Ha12 and Ha41 according to differences they express in their biology.

Received for publication 29 June 1992.

[1] Contribution from the Institut National de la Recherche Agronomique, Centre de Recherches de Rennes.

[2] Nematologist and Statistician, Laboratoire de la Chaire de Zoologie, Domaine de la Motte, BP 29, 35650 Le Rheu, France.

[3] Cytogeneticist, Station de Génétique et d'Amélioration des Plantes.

The authors thank Paulette Pénard and Paulette Abélard for their technical assistance and R. Cook for his helpful comments.

Materials and Methods

Resistance tests: Wheat plants were grown outdoors in 450-ml polyvinylchloride bottles supported by a metallic square case. The bottles, with plastic corks inserted and cut for water drainage, were inverted and the bases were cut off. The bottles were filled with 400 ml of heat-disinfested (24 hours at 80 C) soil mixture (50% loam, 25% sand, 25% compost; 4% organic matter; pH 5.1). *Heterodera avenae* pathotypes Ha12 from Nuisement sur Coole (Marne) and Ha41 from Villasavary (Aude) reared during the previous year on wheat cv. Hardi were used as inoculum. Cysts were contained in 250-μm nylon mesh bags.

In a first experiment (1985–86), the inoculum was 5, 10, 20, or 30 cysts per bottle. The inoculum potential for 10 cysts was estimated from four aliquots as 7.8 ± 0.6 and 9.2 ± 1.1 juveniles/ml soil for pathotypes Ha41 and Ha12. A second experiment in 1988–89 used 10 cysts per bottle, giving an inoculum of 6.3 ± 0.5 juveniles/ml soil for each pathotype.

Soil and cysts were placed in the bottles in November. In both experiments, seeds were individually sown at the beginning of December. Plants were fertilized every two weeks with a liquid complete fertilizer (Substral, V.G.S. Distribution, Nanterre, France) from tillering to flowering stages. Water was supplied in dry periods. The experiments were arranged in a randomized complete block design with 8 and 6 replicates per treatment in 1985–86 and 1988–89, respectively.

Plant materials: In 1985–86, cyst production and fecundity were evaluated on the susceptible wheat cv. Hardi and a wheat line with the added chromosomal pair $6M^v$ (= line m36). In 1988–89, cyst production was analyzed in the five wheat cvs. Castan, Top, Moisson, Hobbit, and Fidel, and the $6M^v$(6D) substitution lines in the five above cultivars. These substitution lines were obtained by crossing the addition line m36 (2n = 44; 42 chromosomes of wheat + $6M^v6M^v$) to Cappelle monosomic 6D provided by A. J. Worland (AFRC, UK). The F_1 plants with 42 chromosomes and double monosomic $6M^v$-6D were selected and backcrossed four times to each of the five named cultivars. At each backcross generation, double monosomic plants were selected after analyzing their meiotic behavior. The substitution lines, in which the $6M^v$ pair of chromosomes were substituted for the 6D pair of wheat, were selected in the selfed progenies of double monosomic $6M^v$-6D plants in the fourth backcross generation. Cytogenetical analysis of their selfed progenies revealed that their chromosomal stability was comparable to that of any wheat cultivar (Jahier, unpubl.).

Nematode counts: In September, newly formed cysts were extracted from soil and roots by elutriation and centrifugal flotation (23). They were collected on a 250-μm mesh sieve, copiously rinsed with tap water, and counted under a stereoscopic microscope. The cyst size (width × length, neck excluded) was measured on a sample of 99 encysted females taken from all the combined cysts from each treatment. The analysis for homogeneity of variance of cyst size by the Cochran test (2) did not show any significant difference between pathotypes ($g = 0.14$, $k = 8$, for Ha41; $g = 0.16$, $k = 8$ for Ha12). After hydrating the cysts for 24 hours and crushing them, fecundity was assessed by counting the viable J2 in a counting cell. Viable and nonviable J2 were visually distinguished (11).

Statistical analysis: Counts of cysts and viable larvae were log(x)-transformed to normalize the distributions and a three-factor analysis of variance was performed with the program STAT-ITCF (8). Means were classified according to the Newman-Keuls test, at $P < 0.05$.

Results

Cyst numbers, 1985–1986: There were no significant interactions between pathotypes, cultivars, and inoculation levels. Pathotype Ha41 gave a significantly higher number of cysts than Ha12, and Hardi supported significantly greater reproduction than line m36 (Table 1). Cyst production increased significantly with increasing inoculation density; the differences be-

TABLE 1. Effects of pathotype, cultivar, and inoculum density on reproduction (cysts per plant) and fecundity (second stage juveniles per cyst) of *Heterodera avenae* on *Triticum aestivum*.

Factors	Cysts per plant	Viable juveniles (J2) per cyst
Pathotype		
Ha41	394 ± 118 a	183 ± 99 a
Ha12	171 ± 91 b	190 ± 103 a
Cultivar		
Hardi	376 ± 106 a	207 ± 107 a
m36	190 ± 105 b	165 ± 95 b
Inoculum†		
5	119 ± 67 a	187 ± 95 ab
10	224 ± 102 b	199 ± 102 a
20	348 ± 120 c	179 ± 105 b
30	440 ± 125 d	180 ± 102 b

Means and standard deviations followed by the same letter are not significantly different ($P < 0.05$) by the Newman-Keuls test.

† Cysts per plant.

tween pathotypes and between host genotypes were seen at all inoculum levels (Fig. 1A).

Cyst fecundity, 1985–1986: Because there was a close relationship ($r = 0.80$; $n = 1584$) between the fecundity and the size of cysts, only the results on fecundity are presented (Table 1). There were significant interactions between pathotypes and inoculum ($P = 0.0001$) and between pathotypes, lines, and inoculum ($P = 0.002$) (Fig. 1B). These interactions were probably caused by aberrant results, principally with line m36 at 10- and 30-cyst inoculum levels. The only statistically significant effect was a lower fecundity of both pathotypes on line m36 ($P < 0.05$).

Reproduction on substitution lines, 1988–89: Again, Ha41 produced more cysts than Ha12 (Table 2). The five germplasms varied somewhat in susceptibility to *H. avenae*. Compared with the recipient germplasms, the substitution lines supported nematode reproduction poorly (−66%). The substitution effect differed according to the recipient, being least effective in Fidel for both pathotypes (Table 3).

DISCUSSION

H. avenae pathotypes Ha12 and Ha41 differed in their cyst-forming ability on susceptible hosts, although their fecundity (juveniles per cyst) did not differ appreciably. This result confirmed previous observations from in vitro experiments (22). Reproductive differences among pathotypes may result from interactions of genotypes with environment, as in *G. pallida* (12,17). The effective inoculum had not been measured throughout the experiment, but it is

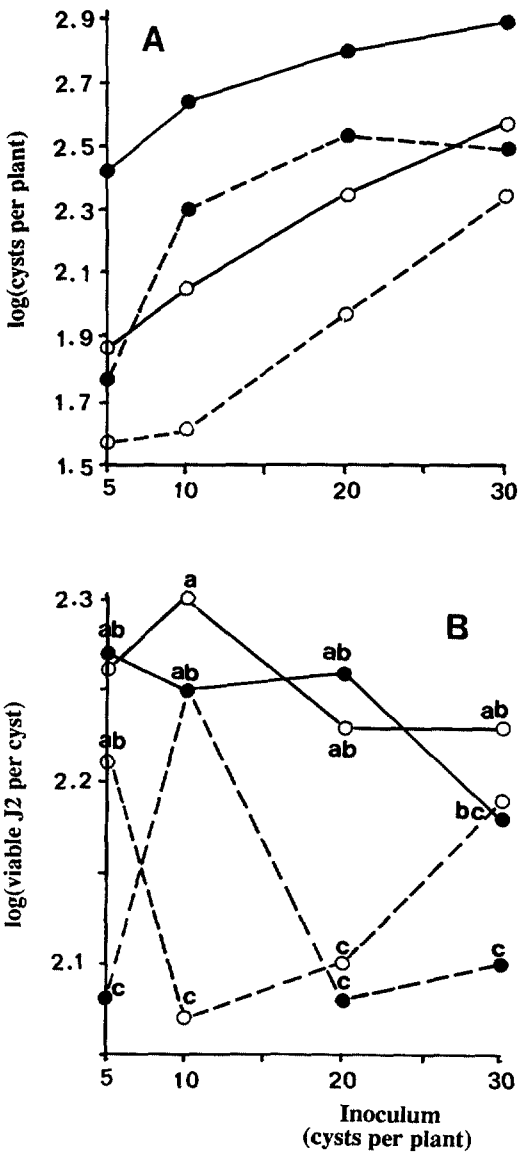

FIG. 1. Reproduction and fecundity of *Heterodera avenae* pathotypes Ha12 (○) and Ha41 (●) on *Triticum aestivum* cv. Hardi (solid lines) or m36 lines (dotted lines) at various inoculum levels. Means followed by the same letter are not significantly different ($P < 0.05$) by the Newman-Keuls test.

TABLE 2. Effects of pathotype, cultivar, and $6M^v$ chromosome substitution on reproduction of *Heterodera avenae* on *Triticum aestivum*.

Factors	Cysts per plant
Pathotype	
Ha41	272 ± 98 a
Ha12	88 ± 37 b
Cultivar	
Castan	236 ± 90 a
Top	222 ± 109 a
Moisson	177 ± 41 ab
Hobbit	155 ± 60 b
Fidel	111 ± 52 b
$6M^v$ substitution	
Cultivars	269 ± 89 a
Substitution lines	92 ± 55 b
Interaction	
Pathotype × Cultivars × Substitution lines	
Ha41/Cultivar	395 ± 116 a
Ha41/Substitution lines	150 ± 77 b
Ha12/Cultivar	143 ± 50 b
Ha12/Substitution lines	33 ± 18 c

Means and standard deviations of data followed by the same letter are not significantly different ($P < 0.05$) by the Newman-Keuls test.

unlikely that the differences in reproduction between Ha12 and Ha41 pathotypes resulted from differences in emergence of juveniles confronted with the growth of the plant root systems. Previous experiments with oat did not demonstrate substantial effects of growth period on cyst production of Ha12 (22). In *H. avenae*, the reproducibility of the reproduction differential (i.e., cysts per plant) between the Ha12 and Ha41 pathotypes could result from genetic differences. Because the southern Ha41 ecotype (located in a Mediterranean climate) is exposed to a harsher environment than the northern Ha12 ecotype (located in an oceanic climate), Ha41 may be more prolific.

The estimation of partial resistance based on cyst counts is generally difficult because of the variation in nematode reproduction. In our experiments, the differences in mean numbers of cysts between susceptible and partially resistant plants were 50% (1986) and 66% (1989). Because of technical constraint, we used fewer replicates than statistically required (7). Nevertheless, the assessment of intermediate resistance originating from $6M^v$ was unaffected. In addition, we rarely observed significant difference between blocks, indicating that the experimental conditions were standardized.

Previous experiments on susceptible and partially resistant potato lines have shown that inoculum density has a positive effect on cyst production and a negative effect on fecundity of *G. rostochiensis* and *G. pallida* (10,16,25). The reproduction of Ha12 and Ha41 pathotypes on Hardi agreed with this finding. In contrast, results with the partially resistant m36 line were obscure.

Intermediate resistance due to the $6M^v$ chromosome was evident in the five substitution lines and differed according to the recipient germplasm. The reduction of numbers of cysts appeared greater in more susceptible backgrounds. The lines carrying the $6M^v$ chromosome are not used at present by wheat breeders because $6M^v$ carries agronomically undesirable genes and does not normally recombine at meiosis with its wheat homoeologues.

To overcome the latter problem, homoeologous recombination was introduced in double monosomic $6M^v$-6D

TABLE 3. Effect of the $6M^v$ (6D) substitution in five wheat germplasms on the reproduction (cysts per plant) of pathotypes Ha12 and Ha41 of *Heterodera avenae*.

	Ha12			Ha41		
	Recipient cultivar	Substitution line	Difference (%)	Recipient cultivar	Substitution line	Difference (%)
Castan	105 ± 37	36 ± 17	−66	525 ± 118	276 ± 147	−47
Top	193 ± 71	32 ± 18	−83	486 ± 200	179 ± 96	−63
Moisson	150 ± 57	28 ± 13	−81	429 ± 51	102 ± 39	−76
Hobbit	201 ± 67	30 ± 14	−85	340 ± 104	50 ± 30	−85
Fidel	67 ± 19	39 ± 29	−42	194 ± 103	144 ± 23	−26

plants homozygous for the ph_1 mutation (24). Cereal cyst nematode resistant recombinants selected from the selfed progenies of these plants could be used in wheat breeding. Varieties with partial resistance such as the $6M^v(6D)$ substitution lines could be useful for integrated management of *H. avenae*. In a rotation program, they could be used alternately with totally resistant cultivars derived, for example, from Loros (21). They could allow limited reproduction of the nematode and thus remove the high pressure for breaking of monogenic resistance.

Literature Cited

1. Andersen, S., and K. Andersen. 1982. Suggestions for determination terminology of pathotypes and genes for resistance in cyst forming nematodes, especially *Heterodera avenae*. EPPO Bulletin 12:379–386.

2. Bowker, A. H., and G. J. Lieberman. 1965. Méthodes statistiques de l'ingénieur. Paris: Dunod Editor.

3. Cook, R. 1974. Nature and inheritance of nematode resistance in cereals. Journal of Nematology 6: 165–174.

4. Cook, R., and K. Evans. 1987. Resistance and tolerance. Pp. 179–231 *in* R. H. Brown and B. R. Kerry, eds. Principles and practice of nematode control in crops. Sydney: Academic Press.

5. Cook, R., and P. A. York. 1982. Resistance of cereals to *Heterodera avenae:* Methods of investigation, sources and inheritance of resistance. EPPO Bulletin 12:423–434.

6. Dosba, F., and R. Rivoal. 1981. Les lignées d'addition blé—*Aegilops ventricosa* Tausch. II.—Etude de leur comportement et de celui de leurs progéniteurs vis à vis d'*Heterodera avenae* Woll. Agronomie 1:559–564.

7. Dosba, F., and R. Rivoal, 1982. Estimation des niveaux de résistance au développement d'*Heterodera avenae* chez les triticinées. Bulletin OEPP 12:451–456.

8. Gouet, H., J. P. Gouet, G. Philippeau, J. Tranchefort, and M. Verneau. 1985. STAT-ITCF. Service des Etudes Statistiques et Informatiques. ITCF-Boigneville.

9. Kort, J., C. P. Jaspers, and D. L. Dijkstra. 1972. Testing for resistance to pathotype C of *Heterodera rostochiensis* and the practical application of *Solanum vernei*-hybrids in the Netherlands. Annals of Applied Biology 71:289–294.

10. LaMondia, J. A., and B. B. Brodie. 1986. Effects of initial nematode density on population dynamics of *Globodera rostochiensis* on resistant and susceptible potatoes. Journal of Nematology 18:159–165.

11. LaMondia, J. A., D. Rawsthorne, and B. B. Brodie. 1986. Methods for reducing experimental variation in *Globodera rostochiensis*. Journal of Nematology 18:415–418.

12. Mugniéry, D., and C. Balandras. 1987. Mise en évidence de différences de balance sexuelle entre populations, chez un phytoparasite amphimictique à déterminisme sexuel épigénique *Globodera pallida* Stone. Comptes Rendus de l'Académie des Sciences de Paris 20:503–506.

13. Mugniéry, D., M. S. Phillips, H. J. Rumpenhorst, A. R. Stone, A. Treur, and D. L. Trudgill. 1989. Assessment of partial resistance of potato to, and pathotype and virulence differences in, potato cyst nematodes. EPPO Bulletin 19:7–25.

14. Nielsen, C. H. 1966. Untersuchungen über die Vererbung der Resistenz gegen die Getriedenematoden (*Heterodera avenae*) beim Weizen. Nematologica 12:575–578.

15. O'Brien, P. C., J. M. Fisher, and A. J. Rathjen. 1980. Inheritance of resistance in two wheat cultivars to an Australian population of *Heterodera avenae*. Nematologica 26:69–74.

16. Phillips, M. S. 1984. The effect of initial population density on the reproduction of *Globodera pallida* on partially resistant potato clones derived from *Solanum vernei*. Nematologica 30:57–65.

17. Phillips, M. S. 1985. Environmental differences and their effect on the assessment of quantitative resistance to potato cyst nematodes. EPPO Bulletin 15:179–183.

18. Rivoal, R. 1977. Identification des races biologiques du nématode à kystes des céréales, *Heterodera avenae* Woll., en France. Annales de Zoologie, Ecologie Animale 9:261–272.

19. Rivoal, R. 1982. Caractérisation de deux écotypes d'*Heterodera avenae* en France par leurs cycles et conditions thermiques d'éclosion. Bulletin OEPP 12:353–359.

20. Rivoal, R., F. Dosba, J. Jahier, and J. S. Pierre. 1986. Les lignées d'addition blé-*Aegilops ventricosa* Tausch. VI.—Etude de la localisation chromosomique de la résistance à l'égard d'*Heterodera avenae* Woll. Agronomie 6:143–148.

21. Rivoal, R., F. Lasserre, M. Hullé, and G. Doussinault. 1991. Evaluation de la résistance et de la tolérance à *Heterodera avenae* chez le blé par des tests miniaturisés. Mededelingen van de Faculteit Landbouwwetenschappen-Rijksuniversiteit, Gent 56: 1281–1292.

22. Rivoal, R., and F. Person-Dedryver. 1982. Caractérisation des pathotypes d'*Heterodera avenae* en France: Influence de la période de culture sur le pouvoir discriminant de cultivars d'*Avena sativa* et différences dans la capacité à former des femelles. Bulletin OEPP 12:387–391.

23. Rivoal, R., F. Person, G. Caubel, and C. Scotto la Massèse. 1978. Méthode d'évaluation de la résistance des céréales au développement des nématodes: *Ditylenchus dipsaci, Heterodera avenae* et *Pratylenchus spp.* Annales de l'Amélioration des Plantes 28:371–394.

24. Sears, E. R. 1977. An induced mutant with homoeologous pairing in common wheat. Canadian Journal of Genetics and Cytology. 19:585–593.

25. Seinhorst, J. W. 1984. Relation between population density of potato cyst nematodes and measured degrees of susceptibility (resistance) of resistant potato cultivars and between this density and cyst content in the new generation. Nematologica 30:66–76.