

Occurrence of a New Russian Wheat Aphid Biotype in Colorado

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ABSTRACT

Russian wheat aphid [RWA, *Diuraphis noxia* (Mordvilko)] is a serious pest of wheat (*Triticum aestivum* L.) in the western USA Great Plains region. While variation in virulence among different RWA isolates has been reported elsewhere, no such variation has been documented among North American RWA isolates. Our objective was to confirm observations in spring 2003 suggesting that a new biotype of RWA was present in southeastern Colorado. The new biotype induced greater injury (leaf rolling and overall plant damage) than the original biotype in standard greenhouse seedling screening tests with a limited collection of resistant and susceptible cultivars. A second experiment with a broader collection of known RWA resistance sources identified only one accession, 94M370 (*Dn7* gene), with resistance to the new biotype. Development of wheat cultivars with resistance to this new biotype will depend on rapid identification and deployment of new resistance sources.

Russian wheat aphid is an important pest of winter wheat and other small grains in Colorado and neighboring states. It was first reported in the USA in 1986 and caused estimated direct and indirect losses of more than \$800 million in the western USA from 1987 to 1993 (Morrison and Peairs, 1998). Additional losses have been incurred since then, primarily in Colorado and surrounding states (Berzonsky et al., 2002). Several effective management approaches have been identified to mitigate damage from RWA (Quisenberry and Peairs, 1998). Russian wheat aphid-resistant cultivars have been widely adopted by Colorado winter wheat producers, particularly in production areas with consistent infestations (Berzonsky et al., 2002). Approximately 25% of the 2003–2004 Colorado winter wheat acreage was planted with resistant cultivars, with a higher proportion found in counties with more consistent RWA pressure (Colorado Agricultural Statistics Service, 2004).

Compared with the greenbug, *Schizaphis graminum* (Rondani), there have been relatively few reports of biotypic variation among different RWA isolates. Puterka et al. (1992) tested eight RWA isolates, including one from the USA, and seven unique virulence patterns were identified. One isolate from the former Soviet Union was virulent to PI 372129, the donor parent of the *Dn4* resistance gene deployed in all but one of the resistant cultivars grown in Colorado. Basky (2003) recently reported virulence of a Hungarian RWA isolate

on wheat lines carrying the *Dn1* (from PI 137739), *Dn2* (from PI 262660), and *Dn4* resistance genes. An isolate of RWA recently identified in Chile (Smith et al., 2004) was shown to be highly virulent to *Dn4*-carrying wheat lines while lines carrying the resistance genes *Dn2*, *Dn5* (from PI 294994), *Dn6* (from PI 243781 or CI 6501), *Dnx* (from PI 220127), and *Dny* (from PI 220350) were resistant to this isolate. In the USA, minor biotypic variation has been identified in RWA collections from the Great Plains, but it has not been considered to be of practical significance as no differential host-plant reactions have been observed (Bush et al., 1989; Shufran et al., 1997).

In late March through May 2003 we received multiple reports of severe RWA infestations and visual plant damage in fields of RWA-resistant 'Prairie Red' (Quick et al., 2001) winter wheat in southeastern Colorado. Infested plants in these fields displayed symptoms characteristic of a susceptible reaction (e.g., white streaking, stunting, and leaf rolling), raising concern that a new RWA biotype was present. In this paper we report the results of two experiments conducted to compare the virulence patterns of our original RWA isolate with a new isolate collected in southeastern Colorado.

MATERIALS AND METHODS

Russian Wheat Aphid Isolation Procedures

Russian wheat aphids were collected from symptomatic Prairie Red winter wheat plants on 29 April 2003 at the Plainsman Research Center approximately five miles north of Walsh, CO (Baca County), and transferred to Fort Collins, CO. A greenhouse colony was established by hand selecting several hundred first-instar RWA from symptomatic tillers infested with the new isolate. These RWA were transferred to pots containing a mixture of wheat and barley plants in screened cages to prevent contamination from our original RWA colony.

Experiment 1

Nine cultivars with known reaction to the original RWA biotype maintained in our screening colony were evaluated with RWA from both the original and the new colony. These included four resistant cultivars carrying the *Dn4* resistance gene from PI 372129 [Quick et al., 1991; 'Ankor' (PI 632275), 'Halt' (PI 584505), Prairie Red (PI 605390), and 'Yumar' (PI 605388)] and 'Stanton' (PI 617033), which carries an uncharacterized RWA resistance gene from PI 220350 (Harvey and Martin, 1990). The four susceptible cultivars included are commonly used as controls in our greenhouse seedling screening procedures: 'Akron' (PI 584504), 'Carson' (PI 501534), 'TAM 107' (PI 495594), and 'Yuma' (PI 559720). Six of the nine cultivars evaluated were essentially three pairs of nearly isogenic lines (NILs) differing for the presence of the *Dn4* RWA resistance gene: Akron-Ankor, Yuma-Yumar, TAM 107-Prairie Red.

Abbreviations: NIL, nearly isogenic line; RWA, Russian wheat aphid.

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Standard greenhouse seedling screening procedures (as described by Nkongolo et al., 1989) were employed to assess differences in virulence between the two RWA isolates. Wheat seedlings were grown in flats under a 16-h photoperiod with daytime temperatures approximately 29 to 32°C and night temperatures approximately 21 to 26°C. The soil mix was composed of six parts Metro Mix (Scotts-Sierra Horticultural Products Company, Marysville, OH), three parts perlite, 1 part sphagnum peat moss, and three parts sieved field soil.

Screened cages were used to separate flats infested with the two isolates. Twelve seeds of each entry were planted without replication in single rows in flats. For both RWA isolates, seedlings were infested at the one-leaf stage, approximately 1 wk after planting, by placing leaf segments containing four to seven RWA at the base of each seedling. Aphid damage was assessed approximately 3 wk after infestation (depending on severity of reaction of susceptible entries) with separate scores for overall plant damage and leaf rolling. Overall plant damage was based on a scale of 1 to 9, with 1 representing apparently healthy plants with small isolated chlorotic spots and 9 representing plants with severe white streaking, chlorosis, stunting, and death (Webster et al., 1987). Leaf rolling was based on a scale of 1 to 3, where 1 represented plants with flat leaves and no apparent rolling and 3 represented plants with tightly rolled leaves (Burd et al., 1993). Under these scales, resistance includes plant damage scores of 1 to 3 and leaf rolling scores of 1, moderate resistance includes plant damage scores of 4–6 and leaf rolling scores of 2, and susceptibility includes plant damage scores of 7 to 9 and leaf rolling scores of 3.

Experiment 2

Evaluation of a broader collection of known resistance sources was undertaken to determine their response to the new isolate. Sixteen genotypes (germplasm accessions, experimental lines, and cultivars) known to be resistant to our original biotype of RWA were evaluated. These genotypes included all known resistance sources designated with a *Dn* gene symbol (*Dn1* through *Dn9*) in addition to several promising resistance sources used by our breeding program since RWA-resistance breeding efforts began in 1987. Plant culture and RWA infestation were done as in Exp. 1, except entries were arranged in a randomized complete block design with three replications. Aphid damage was assessed approximately 3 wk after infestation (depending on severity of reaction of suscepti-

ble entries) with a single plant-damage score based on a scale of 1 to 5, with 1 representing apparently healthy plants with small isolated chlorotic spots and 5 representing severe white streaking, chlorosis, stunting, and death. Under this scale, resistance includes categories 1 and 2, moderate resistance includes category 3, and susceptibility includes categories 4 and 5.

Statistical Analyses

All analyses were conducted using SAS-JMP v. 4.02 (SAS Institute, 2000). For Exp. 1, a matched pairs analysis (paired *t* test) was used to test differences in leaf rolling and plant damage scores between the two RWA isolates across all entries evaluated. Significance of differences in leaf rolling and plant damage scores between groups of resistant and susceptible cultivars was tested by using resistance as a grouping factor in the matched pairs analysis. For Exp. 2, a randomized complete block design ANOVA was conducted. Mean plant damage scores were separated using Fisher's protected LSD.

RESULTS AND DISCUSSION

Experiment 1

A clear difference in virulence was observed between the two isolates of RWA in standard greenhouse seedling screening tests (Table 1). The average scores across all entries in the experiment showed that the new isolate induced significantly greater damage than the original biotype, both for leaf rolling score (2.0 vs. 3.0 score; $P = 0.003$) and plant damage score (4.7 vs. 8.7 score; $P < 0.001$). Group comparisons of the average difference in damage scores between the two isolates for susceptible and resistant groups of entries confirmed this difference, with greater differences in leaf rolling score (0.5 vs. 1.4 difference; $P = 0.048$) and plant damage score (1.8 vs. 5.8 difference; $P < 0.001$) being shown by the resistant group of entries.

The lack of entry replication in this experiment did not permit reliable comparisons to be made between individual entries. The susceptible and resistant pairs of NILs (Akron-Ankor, Yuma-Yumar, TAM 107-Prairie Red), however, clearly responded differently to the original biotype yet were both susceptible to the new isolate.

Table 1. Leaf rolling and plant damage scores for susceptible and resistant wheat cultivars following artificial infestation with original and new isolates of Russian wheat aphid in standard greenhouse seedling screening tests.

Entry	Resistance§	Leaf rolling score†			Plant damage score‡		
		Original isolate	New isolate	Difference	Original isolate	New isolate	Difference
Akron	S	2	3	1	6	9	3
Ankor	R	2	3	1	3	9	6
TAM 107	S	3	3	0	8	9	1
Prairie Red	R	2	3	1	3	8	5
Yuma	S	2	3	1	7	9	2
Yumar	R	1	3	2	2	8	6
Halt	R	1	3	2	2	8	6
Stanton	R	2	3	1	3	9	6
Carson	S	3	3	0	8	9	1
Overall mean		2.0	3.0	1.0	4.7	8.7	4.0
Mean S entries		2.5	3.0	0.5	7.3	9.0	1.8
Mean R entries		1.6	3.0	1.4	2.6	8.4	5.8
<i>P</i> value			0.003¶	0.048#		<0.001¶	<0.001#

† Leaf rolling score rated on a 1 (flat) to 3 (completely rolled) scale.

‡ Plant damage score rated on a 1 (minimal damage) to 9 (severe damage) scale.

§ Resistance reaction to original RWA isolate: R = resistant, S = susceptible.

¶ *P* value for paired *t* test comparing reaction of original and new RWA isolates for all entries tested ($n = 9$ entries).

P value for between-group (e.g., resistant vs. susceptible) comparison of mean difference between reaction to new and original RWA isolates.

This observation, coupled with the comparison between groups of susceptible and resistant entries as mentioned above, confirms that the new isolate of RWA is virulent to the *Dn4* resistance gene used extensively in our breeding program. Additionally, the new isolate appeared to be virulent on Stanton, a cultivar from Kansas State University with resistance derived from a different germplasm source (PI 220350). It is not known, however, whether these two resistance sources are allelic.

Because of the design of this experiment, we were able only to determine relative virulence of the new isolate to genotypes resistant to the original biotype. The data did suggest, however, that the new isolate is more virulent as the onset of symptom development appeared earlier and there was slightly greater damage (2.5 vs. 3.0 leaf rolling score, 7.3 vs. 9.0 plant damage score) within the subset of susceptible entries included in the experiment. Further studies are planned to confirm this observation.

Experiment 2

Significant differences ($P < 0.001$) were observed among entries (Table 2) for plant damage score while variation due to replications was not significant ($P = 0.14$). Most of the entries showed a susceptible reaction (4–5 damage score) while a few showed a moderately resistant reaction (3 damage score). Genotypes carrying the resistance genes *Dn1*, *Dn2*, *dn3*, *Dn5*, *Dn6*, and several promising yet uncharacterized gene sources (including the source in Stanton), were among the entries categorized as susceptible.

The only entry that showed resistance in this experiment was the germplasm accession 94M370. The single dominant resistance gene in this source, denoted as *Dn7* by Marais et al. (1998), was derived through intergeneric transfer from the rye (*Secale cereale* L.) cultivar Turkey 77 to a common wheat carrying the 1BL.1RS wheat–rye translocation (Marais et al., 1994). A recent report by Estakhr and Assad (2002) presented an allelism study

using five known resistance genes (*Dn1*, *Dn2*, *Dn4*, *Dn5*, and *Dn6*) and an Iranian germplasm line denoted as SHZ.W.104. On the basis of their results, the authors proposed the gene symbol *Dn7* for this resistance gene. While the exact origin of SHZ.W.104 is unclear, it is doubtful that it is from the same source as the source transferred from rye.

Previous experiments with 94M370 have shown that it expresses a higher and more consistent level of seedling resistance to the original biotype of RWA than *Dn4*-carrying genotypes from our breeding program (Anderson et al., 2003). Unfortunately, the *Dn7* resistance gene is carried on a 1BL.1RS wheat–rye translocation which has been shown to have serious adverse quality effects for leavened bread products (Graybosch et al., 1990). Visual observations of this source in this experiment and in previous experiments with the original biotype (Anderson et al., 2003) suggest that its mode of resistance is at least in part due to antixenosis, as relatively few RWA successfully colonized the accession despite multiple, repeated artificial infestation attempts.

The exact origin of the new biotype is unknown. It could have originated from a local adaptation to deployed resistance sources or an introduction from areas of the world where greater biotypic diversity of RWA is well documented. In the time period since initial identification of the new biotype in southeastern Colorado, the presence of *Dn4*-virulent RWA populations also have been confirmed in the Nebraska Panhandle and Western Texas (K. Shufran, USDA-ARS, personal communication, 2003). Information on the current and eventual distribution of this new biotype is presently lacking. Until cultivars with resistance to the new biotype are developed, management of RWA infestations in areas of greatest risk will depend on other management approaches, such as biological control, cultural practices, and insecticides.

CONCLUSIONS

We have confirmed the presence of a new biotype of RWA in Colorado. This new biotype is virulent to all RWA resistance sources currently deployed in commercially available cultivars in the western Great Plains region of the USA. This region has shown the greatest risk of economic injury from RWA. The new biotype also is virulent to a broad collection of resistance sources currently in use by breeding programs in the USA and elsewhere. One germplasm line (94M370, *Dn7* gene) showed a highly resistant reaction to the new biotype, yet immediate use of this source in breeding programs may be hampered by adverse quality effects associated with the 1BL.1RS wheat–rye translocation that carries the *Dn7* resistance gene. A systematic search for other resistance sources is currently underway.

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Table 2. Russian wheat aphid damage scores of resistance gene sources and germplasm following infestation with a new isolate of Russian wheat aphid in standard greenhouse seedling screening tests.

Entry	Gene/accession	Reference	Damage score [†]
94M370	<i>Dn7</i>	Marais et al., 1998	1.7
9914BO015	PI 225217	Baker et al., 1998	3.3
GH2002-947-1	<i>dn3</i>	Nkongolo et al., 1989	3.7
<i>Karee-Dn8</i>	<i>Dn8</i>	Liu et al., 2001	3.7
KS94WGRC29	PI 220127	Harvey and Martin, 1990	3.7
STARS-9302W	PI 149898	Baker et al., 1994	3.7
STARS-OK00730	PI 366616	Porter et al., 1993	3.7
CO950043	<i>Dn5</i>	Du Toit, 1988	4.0
CI 6501	<i>Dn6</i>	Harvey and Martin, 1990	4.0
<i>Betta-Dn9</i>	<i>Dn9</i>	Liu et al., 2001	4.0
Stanton	PI 220350	Harvey and Martin, 1990	4.0
CO960293-2	PI 222668	Harvey and Martin, 1990	4.0
KS92WGRC25	Yilmaz-4	Martin and Harvey, 1995	4.0
PI 137739	<i>Dn1</i>	Du Toit, 1987	4.3
PI 262660	<i>Dn2</i>	Du Toit, 1987	4.3
9914BAK007	PI 245462	Porter et al., 1993	4.3
Mean			3.8
LSD (0.05)			0.8
LSD (0.01)			1.0
CV, %			12.3

[†] Damage score rated on a 1 (minimal damage) to 5 (severe damage) scale.

9914BO015, 9914BAK007, and STARS-OK00730 germplasm sources; and Dr. Nora Lapitan (Colorado State University, Fort Collins, CO), for providing seed of the 94M370 germplasm source.

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