Nymphiposition and Population Growth of *Rhoaplosiphum padi* L. (Homoptera: Aphididae) on Conventional Wheat Cultivars and Transgenic Wheat Isolines[1]

L. S. Hesler,[2] Z. Li,[3] T. M. Cheesbrough,[3] and W. E. Riedell

USDA-ARS, Northern Grain Insects Research Laboratory, 2923 Medary Ave., Brookings, SD 57006 USA

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Abstract Nymphiposition and population growth of bird cherry-oat aphid, *Rhoaplosiphum padi* L. (Homoptera: Aphididae), were measured in four experiments with conventional, non-transgenic cultivars of wheat (*Triticum aestivum* L.) and in four separate experiments with isolines of 'Prospect' wheat containing the pin2 gene with promoter for potato proteinase inhibitor II. In two experiments with conventional wheat, population growth of *R. padi* was lower on cultivars 'Sharp,' 'Marshall' and 'Ember' compared to that on 'Russ.' Numbers of *R. padi* were intermediate on '2375' and did not differ from that on other cultivars. In the third and fourth experiments, Sharp and Marshall had less *R. padi* than 'Guard' and 'Prospect,' whereas 'Butte 86' and 'Ivan' had intermediate numbers of *R. padi* that did not differ from that on other cultivars. Nymphiposition by alate *R. padi* did not differ among cultivars, indicating a lack of antixenosis. Transgenic isolines did not show resistance to *R. padi*. Two of three experiments showed no effect of isoline on nymphiposition by *R. padi*, and three of four experiments showed no effect of isoline on final numbers of *R. padi*. However, in one experiment, nymphiposition by *R. padi* was greater on some transgenic isolines than others and, after 13 d, some transgenic isolines had greater populations of *R. padi*. Mechanical wounding of transgenic plants had no effect on nymphiposition or final numbers of *R. padi*. Although wheat cultivars Sharp, Marshall and Ember show promise as sources of antibiosis resistance to *R. padi*, more research is needed to understand potential use of proteinase transgenes in wheat for cereal aphid management.

Key Words *Triticum aestivum*, *Rhoaplosiphum padi*, transgenic wheat, antibiosis, host-plant resistance

Wheat, *Triticum aestivum* L., is attacked by a complex of cereal aphid species (Homoptera: Aphididae), such as *Rhoaplosiphum padi* (L.) (bird cherry-oat aphid), *Diuraphis noxia* (Kurdjumov) (Russian wheat aphid) and *Schizaphis graminum* (Rondani) (greenbug). Winged aphids colonize spring and winter grains, often in the seedling stage, and successive generations of parthenogenetic viviparae develop on grain plants, sometimes to damaging levels (Wallin et al. 1967, Kieckhefer 1975, Araya et al. 1987). Cereal aphids can cause yield loss by decreasing various yield components such as numbers of spikelets and seeds; aphid infestations originating at seedling stage lead to greatest yield loss (Pike and Schaffner 1985, Kieckhefer and Gellner 1992, Kieckhefer et al. 1995). Some species, such as *R. padi*, vector barley yellow

[1]Received 01 June 2004; accepted for publication 01 July 2004.

[2]Address inquiries (email: *lhesler@ngirl.ars.usda.gov*).

[3]Biology-Microbiology Department, South Dakota State University, Brookings, SD 57007.

dwarf virus, which can cause plant disease and further yield loss in wheat (Fitzgerald and Stoner 1967, Gill 1980, Carrigan et al. 1981, Riedell et al. 1999).

Limiting infestations of cereal aphids is a key to preventing yield loss in wheat, and host-plant resistance is one strategy to limit cereal-aphid infestations in wheat (Smith et al. 1999, Webster and Kenkel 1999). Many arthropods such as aphids, whiteflies and mites, initially invade crops in low numbers, but populations increase gradually over many generations before reaching damaging levels. For these arthropods, even low-to-moderate levels of antixenosis and antibiosis can be effective in preventing them from reaching economic damage levels (Dreyer and Campbell 1987, Panda and Khush 1995). Thus, sources of resistance that limit nymphiposition and subsequent population growth of *R. padi* and other cereal aphids may be valuable in reducing crop loss and precluding use of aphicides (Webster and Kenkel 1999).

Efforts to obtain host-plant resistance to cereal aphids include (1) conventional screening and selection of wheat cultivars and closely-related species and (2) use of biotechnology to transfer aphid-resistance genes from unrelated species into wheat cultivars (Panda and Khush 1995, Quisenberry and Clement 2002). Conventional methods have led to development of wheat cultivars with significant resistance to some cereal aphids, such as *D. noxia* or *S. graminum* (Souza 1998, Smith et al. 1999, Quisenberry and Clement 2002). However, few wheat cultivars have been identified with resistance to *R. padi*, and those generally show relatively low levels of resistance (Hesler et al. 1999, 2003, Havlíčková 2001). Because of limited sources of cereal-aphid resistance within wheat, researchers have introduced genes from other plant sources for defense against aphids.

Plant proteinase inhibitors are inducible defense compounds of dicotyledonous plants that are active against a broad spectrum of insects and plant pathogens (Ryan 1989, Boulter 1993). Potato proteinase inhibitors limit development and survival of insects (Lawrence and Koundal 2001). Potato proteinase inhibitors are among the most extensively studied systemic, plant-defense systems against insects and candidates for use in transgenic host-plant resistance (Ryan 1990, Boulter 1993, Lawrence and Koundal 2001). Potato proteinase inhibitors I and II are considered powerful inhibitors of serine proteinases (Johnson et al. 1989). Potato proteinase inhibitor I strongly inhibits chymotrypsin and weakly inhibits trypsin; whereas, potato proteinase inhibitor II actively inhibits both (Johnson et al. 1989). Some monocotyledonous crop plants, such as rice, have been transformed to include potato proteinase inhibitor genes that confer resistance to insect pests such as a stem-boring lepidopteran, *Sesamia inferens* F. (Duan et al. 1996). More importantly for aphid management, Xu et al. (1993) found that potato proteinase inhibitor was expressed in the phloem of transgenic rice plants. Success with rice has spurred research on transgenic insect resistance with other monocots such as wheat.

Previous studies have identified proteinase inhibitors that limit growth and survival of aphids on artificial diets (Rahbe et al. 1995, Tran et al. 1997). Potato proteinase inhibitor I and II were each particularly effective in limiting growth and reproduction of *R. padi, D. noxia*, and *S. graminum* in feeding trials using artificial diet (Tran et al. 1997). If these proteinase inhibitors are as effective against cereal aphids *in planta* as they are *in vitro*, then genes for these proteinases would be suitable for development of transgenic wheat lines that limit cereal-aphid infestations (Tran et al. 1997). Transgenic isolines of wheat expressing potato proteinase inhibitor II have recently been derived using the cultivar 'Prospect' (Li 1999). In this paper, we use laboratory experiments to first characterize performance of *R. padi* on Prospect wheat relative to

conventional wheat cultivars, and we then compare performance of *R. padi* among Prospect wheat and transgenic isolines derived from it.

Materials and Methods

All *R. padi* used in our tests were obtained from a virus-free, multiclonal stock colony maintained on barley, *Hordeum vulgare* L., plants in growth chambers (20°C, photoperiod of 13:11 [L:D] h) at the Northern Grain Insects Research Laboratory, Brookings, SD. The *R. padi* colony was established by collecting aphids from small-grain fields in Brookings Co., SD, in late summer of 1995 (Riedell et al. 1999). We placed field-collected adults in small (2-cm diam, 2-cm long) cages described by Kieckhefer and Derr (1967) that held a 20% sucrose solution in sachets of Parafilm® (American National Can Co., Greenwich, CT) membranes. Caged aphids were checked every few hours, and neonate offspring deposited within the first 30 h were transferred to noninfested plants (Kieckhefer and Gellner 1992). This procedure was repeated once or twice per year with colony aphids to insure they remained free of virus, and occasionally leaf tissue was tested serologically (Agdia, Elkhart, IN) to insure that colony plants were not viruliferous.

We tested nine, contemporary wheat cultivars grown in the northern Great Plains of the United States: 'Marshall' (CItr 17920, Busch et al. 1983), 'Sharp' (PI 540401, Cholick et al. 1992), 'Ember' (PI 612965, Wiersma and Bennett 2001), '2375' (PI 601477, Wiersma and Bennett 2001), 'Russ' (PI 592785, Wiersma and Bennett 2001), 'Butte 86' (ND597), 'Guard' (CItr 17934, Cholick et al. 1984), 'Ivan' (Wiersma and Bennett 2001) and 'Prospect' (PI 491568, Cholick 1990). These cultivars are all spring wheats and were selected for testing because they are widely grown throughout the northern Great Plains. Seeds of each cultivar were obtained from the Spring Wheat Breeding program, South Dakota State University, Brookings.

Transgenic isolines of Prospect wheat that expressed potato proteinase inhibitor II were derived and selected at the Northern Plains Biostress Laboratory, South Dakota State University, Brookings. Details were given by Li (1999). Briefly, isolines were derived by using a modified anther culture system (Yu 1995, Li 1999) developed by Shin (1994). In this system, immature anthers of Prospect wheat were harvested and cultured on a medium that stimulates pollen to divide and produce embryogenic callus tissue. About 3000 calli were derived from anther culture and subjected to transformation. The transformation technique consisted of coating the gene for potato proteinase inhibitor II with promoter (pin2) onto tungsten particles and shooting particles into anther-culture tissue under high-pressure helium acceleration. Prospect wheat was used because it has been widely grown in South Dakota and has no known resistance to cereal aphids (Cholick 1990). About 2500 calli of Prospect wheat regenerated after being subjected to the transformation technique, producing almost 300 putative transgenic plants. These putatively transgenic plants were pooled into groups of about 10 and screened for potato proteinase inhibitor II using techniques of Michelmore et al. (1991), with non-transformed Prospect used as a control. Individual plants from positive pools were re-screened. Transformed plants were allowed to self-pollinate, and 5 R_1 offspring of each transformed plant were tested for gene expression of potato proteinase inhibitor II by mechanical wounding with a hemostat. Twenty-four hours later, RNA was collected from each wounded plant and used for reverse transcriptase-PCR (RT-PCR; Dresselhaus et al. 1996) with Ppi-II primers. Resulting products were fractioned at 75V on a 1.5% agarose gel. Bands were re-

moved, eluted and subjected to automated DNA sequencing with an ABI Prism 310 genetic analyzer (Applied Biosystems; Foster City, CA). This resulted in 17 isolines that could express potato proteinase inhibitor II. In addition, plants of isoline '030-1,' which had been subjected to the transformation procedure but did not express potato proteinase inhibitor II, were used along with plants of non-transformed Prospect wheat as controls.

We tested conventional cultivars and transgenic isolines of wheat in a total of eight experiments. The first four experiments involved conventional wheat cultivars, and the latter four involved Prospect wheat and transgenic isolines derived from it. Experiments measured the performance of *R. padi* in terms of number of nymphs deposited within 24 h of infesting with winged adults (except experiment 5, see below) and total number of *R. padi* on plants 13 d after infestation. Counts of nymphs that were made 24 h after infestation tested cultivar suitability for nymphiposition, and counts 13 d after infestation evaluated cultivars for adverse effects on *R. padi* population development.

All experimental plants were prepared by germinating seeds between layers of moist paper towels held in plastic containers in the dark (Hesler et al. 1999). After 48 h at 20°C, we planted individual seedlings exhibiting uniform root and coleoptile growth in cylindrical tubes (D40 Deepot Cell, 6.4 cm diam, 25.0 cm ht.; Stuewe and Sons, Corvalis, OR) filled with a 2:1:1 mixture of Vienna soil (fine-loamy, mixed Calcic Hapludolls), coarsely ground coconut shells, and perlite, or a 2:1:1 mixture of Vienna soil, peat, and vermiculite (fifth experiment only). All experiments were run in a growth chamber at 20°C, approximately 50% R.H., and 13:11 (L:D) photoperiod. In each experiment, 7-d-old plants (first leaf unfurled, second leaf emerging) were infested with *R. padi* and then covered with vented, clear plastic cylinders (3.5 cm diam, 35 cm ht.).

The first four experiments with conventional cultivars were split into two sets due to labor constraints. The first set included cultivars Marshall, Sharp, Ember, 2375 and Russ, and the second set included Marshall, Sharp, Butte 86, Guard, Ivan and Prospect. For each set, Sharp was used as the control, as previous research indicated its susceptibility to yield loss from *R. padi* infestations (Kieckhefer et al. 1995). Marshall was included in the second set of experiments as a positive control in light of reduced *R. padi* population growth on it in the first set of experiments.

We infested individual plants with three winged *R. padi*, counted *R. padi* nymphs per plant 24 h later (day-1 counts), standardized populations to 5 neonates, and counted all aphids per plant 13 d after infesting. Each experiment was arranged with randomized complete block design, and had 11 to 12 replications for nymphiposition tests. The first 8 replicates having 5 or more nymphs per plant on day 1 were retained through day 13. Because conditions and procedures for experiments 1 and 2 were virtually identical, data for day-1 nymphal counts from experiments 1 and 2 were combined into one data set, and data for day-13 counts of these two experiments were combined into a separate set. Data sets for experiments 3 and 4 were similarly combined. Day-1 and day-13 counts for each pair of experiments were subjected to separate analyses of variance. We used a mixed model for statistical analysis (PROC MIXED; Littell et al. 1996), with cultivar as a fixed factor, experiment and block as random factors, and block nested within experiment. Cultivar means were estimated by calculating the least square means and separated by using a least square means procedure (LSMEANS feature; Littell et al. 1996).

Experiments 5 through 8 used only isolines of Prospect wheat, with Prospect and isoline 030-1 included as controls in each experiment. Experiment 5 was an initial test

of *R. padi* population growth among all 17 isolines that expressed potato proteinase inhibitor II. We used apterous *R. padi* for infesting isolines because we could more reliably produce large numbers of good quality apterae than alatae for infesting all replicates of the 19 isolines (including controls). Isolines were evaluated by counting aphids 7 d after infesting each plant with 5 wingless, late-instar or adult *R. padi*. Counts from day 7 evaluated cultivars for adverse effects on *R. padi* population development, and these counts were subjected to one-way analysis of variance using a general linear model procedure (PROC GLM, SAS Institute 1988).

In experiments 6 and 7, nine of the isolines that qualitatively indicated highest amounts of gene expression according to RT-PCR were re-tested. The nine isolines were divided among experiment 6 (five isolines) and experiment 7 (four isolines) due to labor constraints. Isolines 001-3, 003-3, 004-2, 015-3 and 016-3 were tested in experiment 6, and isolines 021-1, 021-2, 021-4 and 030-2 were tested in experiment 7. Individual plants were infested with three winged *R. padi*. We counted all nymphs deposited through day 1, thinned infestations to five neonates per plant, and counted all aphids per plant on day 13. In these two experiments, isoline was the sole treatment factor, and day-1 and day-13 aphid counts from individual experiments were each subjected to separate, one-way analyses of variance (PROC GLM, SAS Institute 1988), with a significant outcome followed by Fisher's (1935) protected LSD test to separate isoline means. When both nymphiposition and population varied by isoline in an experiment, linear and rank correlation tests were performed to determine if there was any relation between the two dependent variables on the basis of individual host plants or isolines, respectively (PROC CORR, SAS Institute 1988).

Experiment 8 was run to determine if mechanical wounding shortly before *R. padi* infestation would affect subsequent nymphiposition and aphid population growth, as potato proteinase inhibitor was expressed within 24 h after we had mechanically wounded plants of transgenic isolines. This experiment was set up in 2-by-4 factorial design, with two wounding levels (wounded, not wounded) and four isolines (004-2, 021-4, 030-1, and Prospect). Twenty, 6-day-old R_2 plants of each isoline were used. Ten plants from each isoline were subjected to wounding of the tip of the oldest leaf by hemostat 24 h before infestation. The remaining 10 plants were not wounded by hemostat. Each plant was then infested with three winged *R. padi*. Nymphs were counted 24 h after infesting (day 1 counts), thinned to 5 per plant, and allowed to grow and reproduce until day 13, when all aphids were counted. Aphid counts made at one and 13 d after infesting were each subjected to a factorial analysis of variance using a general linear model procedure (PROC GLM, SAS Institute 1988).

Results

Conventional wheat cultivars. Nymphiposition by alate *R. padi* did not differ ($P > 0.05$) among cultivars in experiments 1 and 2 ($\bar{x} \pm$ SE = 10.9 ± 0.6 nymphs per plant; $F = 0.78$; df = 4, 76.1) or in experiments 3 and 4 ($\bar{x} \pm$ SE = 6.7 ± 0.2 nymphs per plant; $F = 0.98$; df = 5, 105). However, counts of *R. padi* on day 13 differed among cultivars (experiments 1 and 2: $F = 11.48$; df = 4, 61.9; $P < 0.0001$; experiments 3 and 4: $F = 7.10$; df = 5, 70.4; $P < 0.0001$). In experiments 1 and 2, there were less *R. padi* on Marshall and Sharp than on Russ and 2375; whereas, the number on Ember was intermediate but less than that on Russ (Table 1). In experiments 3 and 4, there were less *R. padi* on Marshall and Sharp than on Guard or Prospect; whereas, number of *R. padi* on Ivan and Butte 86 was intermediate to these four cultivars (Table 1).

Table 1. Numbers of *Rhoaplosiphum padi* among wheat cultivars

Accession	Mean no. per plant ± SE	
	Experiments 1 and 2	Experiments 3 and 4
Marshall	95.9 ± 4.4 a	111.7 ± 5.9 a
Sharp	102.9 ± 3.7 a	116.8 ± 5.4 a
Ember	109.1 ± 7.9 ab	–
2375	130.1 ± 7.8 bc	–
Russ	133.0 ± 5.7 c	–
Butte 86	–	134.1 ± 7.8 ab
Guard	–	143.3 ± 5.5 b
Ivan	–	133.1 ± 4.1 ab
Prospect	–	149.9 ± 6.9 b

Means ± SE within a column not followed by the same letter are significantly different (LSD test, $\alpha = 0.05$). Dash indicates entry not tested.

Transgenic isolines. In experiment 5, isolines did not differ from Prospect or from one another in number of *R. padi* per plant ($\bar{x}$ ± SE = 132.3 ± 3.9 per plant; $F = 1.59$; df = 18, 126; $P = 0.07$). In experiment 6, lines did not differ ($P > 0.05$) from one another in number of *R. padi* nymphs on day 1 ($\bar{x}$ ± SE = 10.5 ± 0.7 per plant; $F = 1.19$; df = 6, 59) or in final number of *R. padi* on day 13 ($\bar{x}$ ± SE = 189.0 ± 5.9 per plant; $F = 1.92$; df = 6, 38). However, in experiment 7, number of nymphs deposited on day 1 ($F = 3.45$; df = 5, 49; $P = 0.01$) and number of *R. padi* on day 13 differed ($F = 3.64$; df = 5, 32; $P = 0.01$) among isolines. *Rhopalosiphum padi* deposited more nymphs on isolines 021-1 and 030-1 than on Prospect or isoline 030-2, and deposited an intermediate number of nymphs on isolines 021-2 and 021-4 (Table 2). On day 13, all isolines had more *R. padi* than Prospect (Table 2). The numbers of *R. padi* nymphs deposited on day 1 were not correlated ($P > 0.05$) with population numbers on day 13 on the basis of individual test plants ($r_P = 0.18$, n = 48) or isolines ($r_s = 0.77$, n = 6). In experiment 8, isoline and wounding had no effect ($P > 0.05$) on nymphiposition by *R. padi* on day 1 ($\bar{x}$ ± SE = 12.6 ± 0.7 nymphs per plant; wounding, $F = 1.89$; df = 1, 59; isoline, $F = 2.00$; df = 3, 59; interaction, $F = 1.41$; df = 3, 59; $P = 0.25$) or on number of *R. padi* per plant on day 13 ($\bar{x}$ ± SE = 137.3 ± 2.8; wounding $F = 0.02$; df = 1, 46; isoline, $F = 1.57$; df = 3, 46; interaction, $F = 1.03$; df = 3, 46).

Discussion

The conventional, non-transgenic wheat cultivars Marshall, Sharp and Ember were resistant to *R. padi*; whereas, none of the transgenic wheat isolines showed resistance. Population growth of *R. padi* on cultivars Marshall, Sharp and Ember was lower relative to that on other cultivars tested. Antibiosis can be defined as adverse effect(s) of a resistant plant on aphid population development (Scott et al. 1990, Panda and Khush 1995, Kindler et al. 1999), and, thus, our results showed that Marshall, Sharp and Ember exhibited antibiosis resistance to *R. padi*. The greatest

Table 2. Numbers of *Rhoaplosiphum padi* on transgenic isolines of wheat, experiment 7

Isoline	Mean no. per plant ± SE	
	Nymphs* (Day 1)	All *R. padi*** (Day 13)
Prospect†	7.5 ± 1.0 a	142.4 ± 15.8 a
021-1	12.9 ± 1.0 d	194.6 ± 6.4 b
021-2	9.0 ± 1.5 abc	189.9 ± 20.7 b
021-4	11.1 ± 1.4 bcd	204.0 ± 11.9 b
030-1†	11.4 ± 1.6 cd	205.0 ± 8.1 b
030-2	7.8 ± 0.9 ab	190.8 ± 20.2 b

Means ± SE within each column not followed by the same letter are significantly different (LSD test, $\alpha = 0.05$).
* Number of nymphs deposited by 3 alate *R. padi* during a 24-h period.
** Numbers of *R. padi* after 13 d following standardization to 5 nymphs per plant on day 1.
† Non-transformed control.

reductions in *R. padi* numbers occurred on cultivar Marshall. These reductions of 25 to 28% were relatively modest, but small reductions can be important in limiting aphid infestations, especially on seedling plants (Dreyer and Campbell 1987, Panda and Khush 1995). Antibiosis may reduce rate of population increase by limiting reproduction and survival and prolonging generation time (Panda and Khush 1995), and additional testing is needed to determine the degree to which reproduction, survival, and generation time of *R. padi* are affected by Marshall, Sharp and Ember. Also, Scott et al. (1990) suggested that antibiosis experiments that measure aphid population growth might reflect aphid population growth under field conditions, and testing of Marshall, Sharp and Ember in the field is needed to determine relevance of their antibiosis against natural infestations of *R. padi.*

An examination of the pedigrees of Marshall, Sharp and Ember reveals that Marshall's pedigree (Waldron/Era; Busch et al. 1983) differs distinctly from that of Sharp (Butte*2// Fletcher/CItr 13990; Cholick et al. 1992) and Ember (Guard/Sharp// Grandin). 'Butte' is a common ancestor of Sharp, Ember, Butte 86 and Prospect (Cholick et al. 1984), and, thus, it is unlikely to be a source of resistance. Future studies should evaluate 'Waldron' and 'Era' (parental lines of Marshall) and 'Fletcher' and CItr 13990 (ancestors of both Sharp and Ember) for *R. padi* resistance.

Other modalities of resistance may not be operative in the conventional wheat cultivars. For instance, nymphiposition by alate viviparae of *R. padi* did not differ among conventional wheat cultivars in our tests, indicating equal host acceptance or, conversely, a lack of antixenosis. Tolerance, the third form of plant resistance to insects, was not tested among wheat cultivars in our study. However, Kieckhefer et al. (1995) showed that Sharp, Guard and 'Grandin' wheat suffered comparable yield loss from controlled infestations of *R. padi*, indicating that tolerance was not a form of resistance in those cultivars. Ember was derived from crosses of these three cultivars (i.e., Guard/Sharp//Grandin) and, thus, is not expected to show tolerance to *R. padi*

infestation. Information about tolerance to *R. padi* in other cultivars in our study is lacking. Future studies should test for this.

Transgenic wheat isolines showed no resistance to *R. padi*, and in one experiment nymphiposition and population growth were actually greater on transformed isolines. Because greater numbers of *R. padi* occurred in only one of four experiments, we suspect the result might have been due to chance groupings of plants that varied in their suitability to *R. padi*, groupings of aphids that differed in fecundity, or both. Alternatively, the transformation procedure might have caused unwitting changes that improved the nutritive value of some isoline plants to *R. padi*, compromised their intrinsic aphid-defense mechanisms, or both. The lack of correlation between number of *R. padi* nymphs deposited and subsequent population growth suggests that any factors involved in host acceptance for nymphiposition were not necessarily related to or the same as those factors that affected population growth.

The results of our experiments of nymphiposition and population growth of *R. padi* on transgenic isoline plants contrast with results from *in vitro* experiments in which *R. padi* survival and reproduction were reduced on artificial diets containing potato proteinase inhibitor II or other proteinase inhibitors (Tran et al. 1997). Feeding by *R. padi* on our transgenic isolines may have been insufficient to induce expression of potato proteinase inhibitor II at levels that would limit *R. padi* population growth. Mechanical wounding induced potato proteinase activity in our transgenic isolines (and this was used to select expressive isolines). However, failure of transgenic lines to reduce nymphiposition or population growth of *R. padi*, even when infestation shortly followed mechanical wounding, suggests that potato proteinase II is not expressed in sufficient concentrations at phloem-tissue feeding sites to limit *R padi*.

Other studies have found that aphid performance is unaffected or improved on plants transformed to express proteinase inhibitors. Ashouri et al. (2001) found that *Macrosiphum euphorbiae* (Thomas) have shortened developmental time and higher fecundity on transgenic potato plants expressing a cysteine proteinase inhibitor, oryzacystatin I, than on other potato lines. Also, Cowgill et al. (2002) found that *Myzus persicae* L. were unaffected on transgenic potato plants expressing oryzastatin I or chicken egg white cystatin, even though survival and growth of this aphid declined on artificial diets containing either compound. They suggested that lack of activity by chicken egg white cystatin against *M. persicae* on transgenic potato plants was due to its insufficient expression in the phloem, but they did not offer explanation for lack of oryzastatin activity against *M. persicae*. The contrasting results with *M. persicae* between artificial diet studies and plant studies is analogous to the contrasting performance of *R. padi* on artificial diets with proteinase inhibitor (Tran et al. 1997) and that on transgenic plants in our study. Taken together, these results suggest that expression of resistance transgenes in relation to aphid performance is not necessarily straightforward, and that more research is needed to improve understanding of transgene expression for effective use against aphids in wheat and other crops. Consideration of the potential value of transgenes for aphid management should be balanced with concerns about possible negative effects of using transgenic crops in agricultural systems (van Emden 1999, Hunter 2000).

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References Cited

Araya, J. E., J. E. Foster and S. E. Cambron. 1987. A study of the biology of *Rhoaplosiphum padi* (Homoptera: Aphididae) in winter wheat in northwestern Indiana. Great Lakes Entomol. 20: 47-50.

Ashouri, A., D. Michaud and C. Cloutier. 2001. Unexpected effects of different potato resistance factors to the Colorado potato beetle (Coleoptera: Chrysomelidae) on the potato aphid (Homoptera: Aphididae). Environ. Entomol. 30: 524-532.

Boulter, D. 1993. Insect pest-control by copying nature using genetically engineered crops. Phytochem. 34: 1453-1466.

Busch, R., D. McVey, V. Youngs, R. Heiner and F. Elsayed. 1983. Registration of 'Marshall' wheat. Crop Sci. 23: 187.

Carrigan, L. L., H. W. Ohm, J. E. Foster and F. L. Patterson. 1981. Response of winter wheat cultivars to barley yellow dwarf virus infection. Crop Sci. 21: 377-380.

Cholick, F. A. 1990. Prospect wheat. Crop Sci. 30: 233.

Cholick, F. A., G. W. Buchenau and B. G. Farber. 1992. Registration of Sharp wheat. Crop Sci. 32: 282.

Cholick, F. A., D. K. Steiger, J. H. Hatchett, K. M. Sellers, G. W. Buchenau and D. L. Keim. 1984. Registration of Guard wheat. Crop Sci. 24: 1215.

Cowgill, S. E., C. Wright and H. J. Atkinson. 2002. Transgenic potatoes with enhanced levels of nematode resistance do not have altered susceptibility to nontarget aphids. Molecular Ecol. 11: 821-827.

Dresselhaus, T., C. Kagel, H. Lorz and E. Kranz. 1996. Isolation of a full-length cDNA encoding calreticulin from a PCR library of in vitro zygotes of maize. Plant Mol. Biol. 31: 23-34.

Dreyer, D. L. and B. C. Campbell. 1987. Chemical basis of host-plant resistance to aphids. Plant Cell Envir. 10: 353-361.

Duan, X., X. Li, Q. Xue, M. Abo-el-Saad and R. Wu. 1996. Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. Nature Biotech. 14: 494-498.

Fisher, R. A. 1935. The design of experiments, 1st ed. Oliver and Boyd, Edinburgh, Scotland.

Fitzgerald, P. J. and W. N. Stoner. 1967. Barley yellow dwarf studies in wheat (*Triticum aestivum* L.). I. Yield and quality of hard red winter wheat infected with barley yellow dwarf virus. Crop Sci. 7: 337-341.

Gill, C. C. 1980. Assessment of losses on spring wheat naturally infected with barley yellow dwarf virus. Plant Dis. 64: 197-203.

Havlíčková, H. 2001. Screening of productive Czech winter wheat cultivars on resistance to aphids. Rost. Vyroba. 47: 326-329.

Hesler, L. S., W. E. Riedell, R. W. Kieckhefer, S. D. Haley and R. D. Collins. 1999. Resistance to *Rhoaplosiphum padi* (Homoptera: Aphididae) in wheat germplasm cultivars. J. Econ. Entomol. 92: 1234-1238.

Hesler, L. S., W. E. Riedell, R. W. Kieckhefer and S. D. Haley. 2003. Responses of *Rhoaplo-*

siphum padi (Homoptera: Aphididae) on cereal aphid-resistant wheat cultivars. J. Agric. Urban Entomol. 19: 133-140.

Hunter, M. D. 2000. Between hyperbole and hysteria: entomological issues and the deployment of transgenic plants. Agric. Forest Entomol. 2: 77-84.

Johnson, R., J. Navarez, G. An and C. Ryan. 1989. Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defense against *Manduca sexta* larvae. Proc. Natl. Acad. Sci. USA 86: 9871-9875.

Kieckhefer, R. W. 1975. Field populations of cereal aphids in South Dakota spring grains. J. Econ. Entomol. 68: 161-164.

Kieckhefer, R. W. and R. F. Derr. 1967. Rearing three species of cereal aphids on artificial diets. J. Econ. Entomol. 60: 663-664.

Kieckhefer, R. W. and J. L. Gellner. 1992. Yield losses in winter wheat caused by low-density cereal aphid populations. Agron. J. 84: 180-183.

Kieckhefer, R. W., J. L. Gellner and W. E. Riedell. 1995. Evaluation of the aphid-day standard as a predictor of yield loss caused by cereal aphids. Agron. J. 87: 785-788.

Kindler, S. D., K. B. Jensen and T. L. Springer. 1999. Detection and characterization of mechanisms of resistance to Russian wheat aphid (Homoptera: Aphididae) in crested wheatgrass. J. Agric. Urban Entomol. 16: 129-139.

Lawrence, P. K. and K. R. Koundal. 2001. Plant proteinase inhibitors in control of phytophagous insects. Electronic J. Biotechnol. 5: 93-109.

Li, Z. 1999. Aphid resistant gene transformation of wheat by biolistics. M.S. Thesis, South Dakota State Univ., Brookings, 68 pp.

Littell, R. C., G. A. Milken, W. W. Stroup and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Inc., Cary, NC.

Michelmore, R. W., I. Paran and R. V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. 88: 9828-9832.

Panda, N. and G. S. Khush. 1995. Host plant resistance to insects. CAB International, Oxon, United Kingdom.

Pike, K. S. and R. L. Schaffner. 1985. Development of autumn populations of cereal aphids, *Rhoaplosiphum padi* (L.) and *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) and their effects on winter wheat in Washington state. J. Econ. Entomol. 78: 676-680.

Quisenberry, S. S. and S. L. Clement. 2002. Conservation and use of global plant genetic resources for insect resistance. Aust. J. Agric. Res. 53: 865-872.

Rahbe, Y., N. Sauvion, G. Febvay, W. J. Peumans and A. M. R. Gatehouse. 1995. Toxicity of lectins and processing of ingested proteins in the pea aphid, *Acyrthosiphon pisum.* Entomol. Exp. Appl. 76: 143-155.

Riedell, W. E., R. W. Kieckhefer, S. D. Haley, M. A. C. Langham and P. D. Evenson. 1999. Winter wheat responses to bird cherry-oat aphids and barley yellow dwarf infection. Crop Sci. 39: 158-163.

Ryan, C. A. 1989. Proteinase inhibitor gene families: strategies for transformation to improve plant defenses against herbivores. BioEssays 10: 20-24.

1990. Proteinase inhibitors in plants: genes for improving defenses against insects and pathogens. Annu. Rev. Phytopath. 28: 425-449.

SAS Institute. 1988. SAS Procedures Guide, 6.03 ed. SAS Institute Inc., Cary, NC.

Scott, R. A., W. D. Worrall and W. A. Frank. 1990. Comparison of three techniques for measuring antibiosis to the Russian wheat aphid. Southwest. Entomol. 15: 439-446.

Shin, H-K. 1994. Anther culture of selected South Dakota spring wheat (*Triticum aestivum* L.) breeding stock. Ph.D. Diss., South Dakota State Univ., Brookings, 96 pp.

Smith, C. M. 1989. Plant resistance to insects. Wiley, New York.

Smith, C. M., S. S. Quisenberry and F. du Toit. 1999. The value of conserved wheat germ-

plasm evaluated for arthropod resistance, Pp. 25-49. *In* S. L. Clement and S. S. Quisenberry [eds.], Global plant genetic resources for insect-resistant crops. CRC Press, Boca Raton, FL.

Souza, E. E. 1998. Host plant resistance to the Russian wheat aphid (Homoptera: Aphididae) in wheat and barley, Pp. 122-147. *In* S. S. Quisenberry and F. B. Peairs [eds.], Proc. Thomas Say Publ. in Entomol, Entomol. Soc. Am., Hyattsville, MD.

Tran, P., T. M. Cheesbrough and R. W. Kieckhefer. 1997. Plant proteinase inhibitors are potential anticereal aphid compounds. J. Econ. Entomol. 90: 1672-1677.

van Emden, H. F. 1999. Transgenic host plant resistance to insects—some reservations. Ann. Entomol. Soc. 92: 788-797.

Wallin, J. R., D. Peters and L. C. Johnson. 1967. Low-level jet winds, early cereal aphid and barley yellow dwarf detection in Iowa. Plant Dis. Reporter 51: 528-530.

Webster, J. A. and P. Kenkel. 1999. Benefits of managing small-grain pests with plant resistance, Pp. 87-114. *In* B. R. Wiseman and J. A. Webster [eds.], Economic, environmental, and social benefits of resistance in field crops. Entomol. Soc. Am., Lanham, MD.

Wiersma, J. J. and J. M. Bennett [eds.]. 2001. The small grains field guide. Univ. Minnesota Extension Service, St. Paul, 160 pp.

Xu, D., D. McElroy, R. W. Thornburg and R. Wu. 1993. Systemic induction of a potato pin2 promoter by wounding, methyl jasmonate, and abscisic acid in transgenic rice. Plant Mol. Biol. 22: 573-588.

Yu, R. 1995. Rapid selection for tan spot resistance through anther culture of spring wheat (*Triticum aestivum* L.) F_1 hybrids. Ph.D. Diss., South Dakota State Univ., Brookings, 125 pp.

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Yee, W. L. 2005. Seasonal distributions of eggs and larvae of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) in cherries. J. Entomol. Sci. 40: 158-166.

The first line of the article is printed in error and should read, "The western cherry fruit fly, *Rhagoletis indifferens* Curran, is the major pest of sweet cherries, *Prunus avium* (L.) L., in the Pacific Northwest of the United States."

Hesler, L. S., Z. Li, T. M. Cheesbrough and W. E. Riedell. 2005. Nymphiposition and population growth of *Rhopalosiphum padi* L. (Homoptera: Aphididae) on conventional wheat cultivars and transgenic wheat isolines. J. Entomol. Sci. 40: 186-196.

The generic name for the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Homoptera: Aphididae), was inadvertently misspelled throughout the article.

Asaro, C., C. W. Berisford, M. J. Dalusky, J. L. McLaughlin, and C. Czokajlo. 2005. Preliminary tests of an attracticide formulation for control of the Nantucket pine tip moth (Lepidotera: Tortricidae). J. Entomol. Sci. 40: 240-245.

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