Hessian Fly (Diptera: Cecidomyiidae) Interactions With Barley, Rice, and Wheat Seedlings

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ABSTRACT   A choice test revealed that Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), adults deposited ≈3 times more eggs on wheat (*Triticum* spp.), seedlings than on barley (*Hordeum* spp.) or rice, *Oryza sativa* L., seedlings. On a barley seedling, 49.4% of eggs were deposited on either the abaxial leaf surface or the coleoptile and first leaf sheath (C&FLS), where newly hatched larvae die due to their inability to migrate into the interspace between leaf sheaths. In comparison, only 14% of eggs were deposited on the abaxial leaf surface or C&FLS on a wheat seedling. The average death rate of Hessian fly larvae in seedlings of an apparently susceptible barley line was 60%, compared with only 10% in seedlings of a susceptible wheat cultivar. The development of Hessian fly larvae was also much slower in barley seedlings than in wheat seedlings. It took 12 d for Hessian fly larvae to finish the first and second instars in susceptible barley seedlings, compared with 10 d in susceptible wheat seedlings under the same conditions. These results indicate that barley is not a good host for the Hessian fly. Our results also confirmed that rice is a nonhost for the Hessian fly. The resistance mechanism in rice was different from that in *R* gene resistant wheat. Hessian fly larvae grew a little and died more slowly in rice seedlings, whereas Hessian fly larvae died quickly without growth in resistant wheat.

KEY WORDS   Hessian fly, barley, rice, wheat, host plant resistance

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is one of the most destructive pests of wheat (*Triticum* spp.) in the United States, western Asia, and northern Europe (Hatchett et al. 1987, Pauly 2002, Harris et al. 2003). Resistance (*R*) genes in wheat have been the most effective means for controlling Hessian fly damage. To date, 33 *R* genes or alleles have been identified from wheat or wheat relatives, including rye, *Secale cereale* L., and goat grasses (*Aegilops* spp.) (Liu et al. 2005a, Sardesai et al. 2005). Many of these *R* genes have been incorporated into elite wheat genotypes and deployed in agriculture. The challenge for host plant resistance is that the effectiveness of an *R* gene lasts only 6 to 8 yr after its initial deployment before virulence develops in exposed populations (Hatchett et al. 1987, Ratcliffe et al. 2000). Consequently, new *R* genes need to be continuously identified and incorporated into wheat cultivars. In the long run, a better understanding of the molecular mechanisms of host and nonhost resistance is needed

for a more rational design of durable resistance to the Hessian fly.

The Hessian fly also attacks barley (*Hordeum* spp.) (Jones 1936, Kanno and Harris 2000b, Harris et al. 2001). To control Hessian fly damage to barley, breeders are also developing barley cultivars with resistance to the Hessian fly. Several resistant barley lines have been identified (Buntin and Raymer 1992) and the resistance in some of the resistant barley lines seems to be controlled by partially dominant genes (Olembo et al. 1966). Other wheat-related species including rye, triticale, and wild grasses [e.g., *Agropyron repens* (L.) Beauv., *Agropyron repens smithii* (Rydb.), *Elmus virginicus* L., *Elmus canadensis* L., and *Aegilops* spp.] also can serve as alternative hosts for the Hessian fly when wheat is not available (Jones 1938, 1939; Zeiss et al. 1993; Harris et al. 2001, 2003). Rice, which is more distantly related to wheat, has not been evaluated for its suitability as a Hessian fly host. Because wheat and rice are usually grown in different geographic regions under different ecological conditions (rice fields with water), rice is probably a nonhost for the Hessian fly and should be a useful organism for studying nonhost resistance to this insect. Nonhost resistance, which refers to the phenomenon that all members of a plant species exhibit resistance to all members of a given herbivore species (Heath 1991, Thordal-Christensen 2003), is an area that has not been studied extensively in insects and could result in new approaches for more durable host resistance. Our long-term goal is to elu-

cidate the molecular mechanisms linked with host and nonhost resistance. As an initial step, this study examined similarities and differences among Hessian fly interactions with wheat, barley, and rice, *Oryza sativa* L., seedlings.

Materials and Methods

Hessian Fly. Three Hessian fly populations were used in this study: a Kansas population (Scott-KS-05), an Oklahoma population (Kay-OK-06), and an Indiana population. The Indiana population is commonly referred as to biotype L and was provided by Sue Cambron (USDA–ARS, West Lafayette, IN). The Kansas and Oklahoma populations contain a mixture of biotypes with different virulence patterns to known wheat *R* genes (Chen et al. 2009).

Hessian fly populations were maintained on 1.5-leaf seedlings of 'Karl-92', a cultivar susceptible to all Hessian fly biotypes, in growth chambers at 20°C (daytime) and 18°C (nighttime) and a photoperiod of 12:12 (L:D) h. For long-term storage, Hessian fly third-stage (prepupal) larvae and pupae were collected and stored at 4°C.

Plants. Two wheat lines, 'Newton' and 'Molly', were used. Newton is a Hessian fly-susceptible cultivar that does not contain any *R* genes. Molly is an isogenic line derived from Newton (Patterson et al. 1994) and contains the Hessian fly *R* gene *H13* (Liu et al. 2005b). One hundred barley lines were randomly selected from the Barley Genetic Stocks Collection (http://www.ars.usda.gov/Main/docs.htm?docid = 2922), and the seeds of these lines were provided by the National Small Grains Research Facility at Aberdeen, ID. Four rice varieties (three Japonica and one Indica) were randomly selected from the Genetic Stocks–Oryza collection and the seeds of these lines were provided by the Dale Bumpers National Rice Research Center at Stuttgart, AR.

Resistance/Susceptibility Screening. Wheat lines were grown in flats with dividers. Each wheat line had 15–20 plants, which were grown in a growth chamber at 20°C (daytime) and 18°C (nighttime) with a photoperiod of 12:12 (L:D) h. At the 1.5-leaf-stage, plants were caged with a cheesecloth tent and infested with Hessian fly eggs by releasing adult flies into the tent. When the average egg density on the first leaf of a seedling reached approximately eight eggs per plant, infestation was stopped. Three weeks after infestation (equivalent to 16 d after the initial Hessian fly larval attack), resistant and susceptible plants were categorized and recorded. Barley lines were grown and tested in the same way as in wheat.

Rice seeds were germinated in petri dishes. After germination, seeds with young plants were placed on soil in individual pots, and they were grown in a growth chamber set at 32°C (daytime)/30°C (nighttime) with a photoperiod of 12:12 (L:D) h until plants reached the two-leaf stage. The plants were then grown in the same conditions as barley and wheat for an additional 2 d when the plants reach the 2.5-leaf stage. Because the first leaf of rice is very small, the

2.5-leaf stage of rice seedlings is essentially equivalent to the 1.5-leaf-stage of wheat and barley seedlings in terms of leaf area. The plants were then infested with Hessian fly eggs as described previously.

Choice and Nonchoice Tests. For choice tests, barley, rice, and wheat seedlings were grown separately in individual pots. When barley and wheat plants reached the 1.5-leaf-stage and rice plants the 2.5-leaf-stage, the plants were placed in the same growth chamber. Pots containing barley, wheat, or rice seedlings were randomly arranged in a circle with an empty pot in the middle. These pots were then caged together under a cheesecloth tent and were infested with eggs by releasing a fixed number (one mated female per plant) of flies into the empty pot in the middle. This insect/plant ratio resulted in a higher density of eggs than that in a typical resistance/susceptibility screening described previously. After 3 d when all Hessian fly adults were dead, the cheesecloth tent was removed. The number of eggs on each plant was counted immediately after the cheesecloth tent was removed.

For nonchoice tests, plant conditions were the same as for choice tests. However, each pot was caged separately and exposed to three Hessian fly females per plant.

Hessian Fly Establishment, Mortality, and Emergence. The percentages of Hessian fly larvae that hatched and migrated successfully into the interspace between leaf sheaths were determined as follows. Plants were grown in pots and infested with Hessian fly adults as described in nonchoice tests. Three days after infestation, the number of eggs on the adaxial surface of the leaf (the first leaf for barley and wheat plants and the second leaf for rice seedlings) was counted with a hand-held magnifier. At this stage, the second leaf for barley and wheat and the third leaf for rice had just emerged and Hessian fly adults rarely deposited eggs on them. If eggs were found occasionally on the newly emerged leaf, they were destroyed with a needle. The plant was then tagged and put back into the growth chamber to culture for three more days, when all larvae (except dead embryos) were hatched. After that, the plant was dissected and the number of Hessian fly larvae at the interspace between the respective leaf sheathes was counted. The percentage of Hessian fly larvae that hatched and migrated successfully into the interspace between leaf sheaths was calculated by dividing the number of larvae in the interspace with the number of eggs (determined above) for a plant. The mean percentage was the average from 12 plants, which were from six pots with two plants from each pot. The six pots were infested independently.

A Hessian fly larva was considered dead if it showed no movement. To determine whether a larva was able to move, the larva was detached from the plant into water and watched continuously for three seconds under a microscope. If there was no apparent movement within the 3 s, the larva was then poked with a dissection needle. If the larva still showed no movement, the larva was considered dead. The percentage

Table 1. Egg distribution among the adaxial and abaxial leaf surfaces and C&FLS on plant seedlings in a nonchoice test

Plant type	Plant no.	No. eggs on a plant			% on adaxial surface[a]	95% CI for odds P(ad.)/P(ab.)	95% CI for odds P(ab.)/P(C&FLS)	95% CI for odds P(ad.)/P(other)
		Adaxial	Abaxial	C&FLS				
RW	36	70.7 ± 11.5	12.4 ± 2.4	2.6 ± 0.8	82.5 ± 0.68	(5.06, 6.47)	(3.64, 6.29)	(4.31, 5.19)
SW	33	46.0 ± 5.5	5.3 ± 1.0	2.2 ± 0.6	85.9 ± 0.83	(7.14, 10.46)	(1.75, 3.42)	(5.36, 7.01)
RB	31	43.1 ± 7.5	28.2 ± 6.7	15.0 ± 2.4	49.9 ± 0.97	(1.38, 1.70)	(1.63, 2.15)	(0.93, 1.08)[b]
SB	15	48.8 ± 9.7	33.3 ± 9.0	12.5 ± 2.9	51.5 ± 1.33	(1.27, 1.68)	(2.17, 3.26)	(0.96, 1.18)[b]
Rice	24	23.5 ± 4.1	2.4 ± 0.6	5.5 ± 1.5	74.9 ± 1.58	(7.09, 13.8)	(0.29, 0.63)	(2.53, 3.52)

RW, resistant wheat Molly; SW, susceptible wheat Newton; RB, resistant barley CIho 15768; SB, susceptible barley CIho 15229; Rice, Nipponbare; P(ad.) ,P(adaxial); P(ab.), P(abaxial); P(other), P(abaxial+ C&FLS).

[a] Larvae hatched from eggs on adaxial leaf-surface have a chance to complete life cycle. Larvae hatched from other locations migrate into soil and die.

[b] No preference for the categories compared.

of dead larvae was calculated by dividing the number of dead larvae with the total number of larvae from a plant. The mean percentage was the average from 12 plants, which were from six pots with two plants from each pot. The six pots were infested independently. Larval mortality was determined daily right after hatching and before entering prepupal stage (flaxseeds).

The length of a live larva was determined as follows. Larvae were detached from plants into water in a petri dish. The lengths of individual larvae were measured with a microscope (MZ APO, Leica MicrosyC&FLS, Wetzlar, Germany) equipped with an internal micrometer. The growth of larvae was measured daily right after hatching and before becoming prepupae (flaxseeds).

The emergence rate of adults from pupae was determined as follows. Twelve plants containing pupae from six independently infested pots (two plants from each pot) were collected and incubated under standard conditions for adult emergence. After incubation for 20 d (twice as long as needed for adult emergence from pupae), the plants were dissected, and emerged (with an empty pupal case) and unemerged pupae were distinguished and recorded. The emergence rate was calculated by dividing the total number of emerged pupae with the sum of emerged and unemerged pupae.

Statistical Analyses. Tests for the preference of Hessian fly oviposition on wheat, barley, and rice seedlings were carried out according to the pairwise comparison method for multinomial proportions (Gold 1963). The preference of Hessian fly oviposition among the adaxial leaf surface, abaxial leaf surface, and the outer surface of the coleoptile and first leaf sheath (C&FLS) for each plant type was analyzed according to the Goodman's large sample test for log ratios of multinomial proportions (Goodman 1965). The 95% simultaneous confidence intervals were obtained following the same procedure, and then modified using Bonferroni's correction, which allows the 95% confidence intervals (CIs) with a family-wise error rate <0.05 even though multiple intervals were constructed.

Percentages of larval hatching and successful migration and the percentage of adult emergence were analyzed on the basis of the logistic regression in R-software (http://www.r-project.org/) through the *glm* command. A deviance test was used to assess the significance of the model parameters compared with a null model.

Results

Hessian Fly Oviposition on Wheat, Barley, and Rice Seedlings in Choice Tests. Mean numbers of eggs per square centimeter of plant surfaces on resistant wheat (Molly), susceptible wheat (Newton), resistant barley (CIho 15768), susceptible barley (CIho 15229), and rice (Nipponbare) seedlings were 15.2 ± 1.6 (n = 30), 15.3 ± 1.3, 4.8 ± 1.1, 5.4 ± 1, and 4.2 ± 1.7, respectively. The data from all plants showed a strong preference of Hessian fly females to choose wheat seedlings over barley and rice seedlings for oviposition (χ^2 = 7.255, df = 1, P = 0.007). In contrast, there was no difference between resistant and susceptible wheat seedlings (P = 0.986, df = 1, χ^2 = 0.00033) or between resistant and susceptible barley seedlings (P = 0.843, df = 1, χ^2 = 0.0388). There was also no difference between barley and rice seedlings for Hessian fly oviposition (χ^2 = 0.0797, df = 1, P = 0.777).

Hessian Fly Oviposition on Adaxial and Abaxial Surfaces and C&FLS. The location where Hessian fly adults lay eggs determines the fate of their offspring larvae. Only those larvae hatched from eggs on the adaxial leaf surface can migrate into the interspace between leaf sheaths, where the larvae can live and develop. Larvae hatched from eggs elsewhere (the abaxial leaf surface or C&FLS) migrate into the soil and die.

The preference of Hessian fly oviposition on the adaxial leaf surface, abaxial leaf surface, and C&FLS of wheat (Newton), barley (CIho 15229), and rice (Nipponbare) was examined in a nonchoice test (Table 1). Multinomial logistic regression analysis indicated a strong preference for the adaxial leaf surface over abaxial leaf surface and C&FLS on wheat and rice seedlings, but no preference on barley seedlings. For wheat, the 95% simultaneous confidence intervals for the odds (probability) of Hessian fly adults to deposit eggs on the adaxial leaf surface over abaxial leaf surface and C&FLS were 7.1–10.5. This result indicated that Hessian fly adults were 7–11 times more likely to deposit an egg on the adaxial leaf surface than on the abaxial leaf surface and C&FLS. For rice, the 95% simultaneous confidence intervals for the probability

Fig. 1. Larvae hatched and successfully migrated into the interspace between leaf sheaths (H&SM larvae). A notched box contains the middle 50% of a data group; the bold line inside a box represents the median; and the two horizontal lines linked to a box by a vertical, dashed line represent the upper and lower 25% of the data group (excluding outliers). Circles represent outliers. Notches that do not overlap indicate difference in the median percentages between two data groups (Chambers et al. 1983). *P* values are all <0.00001 between rice and barley, rice and wheat, and barley and wheat data groups.

Fig. 2. Typical growth symptoms of the resistant and susceptible wheat and barley seedlings after Hessian fly attacks. RW, SW, RB, and SB represent resistant wheat (Molly), susceptible wheat (Newton), resistant barley (CIho 15768), and susceptible barley (CIho 15229), respectively. Black lines beside the seeding images indicate grow deficit. In resistant wheat and barley seedlings, the second leaf sheath was elongated and visible. In susceptible wheat and barley seedlings, the second leaf sheath was not visible because of the inhibition of its elongation by Hessian fly larvae.

of Hessian fly adults to deposit eggs on the adaxial leaf surface over abaxial leaf surface, and adaxial leaf surface over C&FLS were 7.1–13.8. This indicates again that there was a strong preference (7–14 times more likely) for Hessian fly adults to choose the adaxial leaf surface over abaxial leaf surface and C&FLS for oviposition on rice. For barley, the 95% simultaneous confidence intervals for the probability of Hessian fly adults to deposit eggs on the adaxial leaf surface over abaxial leaf surface and C&FLS were 1.3–1.7, indicating that there was no preference for the adaxial leaf surface, abaxial leaf surface, and C&FLS on barley.

Larval Hatching and Migration on Wheat, Barley and Rice Seedlings. Percentages of larval hatching and successful migration into the interspace (the number eggs counted only on the adaxial leaf surface) between leaf sheaths on wheat, barley, and rice seedlings are shown in Fig. 1. The data from the three different types of plants are significantly different from each other ($\chi^2 = 792.3$, df = 2, $P < 0.0001$). Many neonate larvae on rice were dead on the leaves, but this was not quantified.

Phenotypic Reactions of Wheat, Barley, and Rice Seedlings to Hessian Fly Attacks. Typical phenotypic reactions of resistant and susceptible wheat seedlings 16 d after the initial Hessian fly attack are shown in Fig. 2. When evaluating barley lines for Hessian fly resistance, we found similar phenotypic symptoms among barley seedlings. Some barley seedlings grew relatively normally after Hessian fly attacks, whereas the others were stunted. Accordingly, we classified barley plants into resistant and susceptible categories accord-

ing to the same phenotypic reactions observed in resistant and susceptible wheat seedlings.

Of the 100 barley lines selected randomly, 94 were tested successfully, whereas the other six lines did not germinate. The phenotypic reactions of the 94 barley lines in response to Hessian fly attacks are given in Table 2. Six of the 94 lines (CIho 15478, CIho 15768, CIho 5041, CIho 6804, PI 10585, and PI 552963) exhibited 100% resistant plants, whereas four of the lines (CIho 12119, CIho 15229, CIho 15549, and GSHO 934) exhibited 100% susceptible plants. The other 84 lines exhibited heterogeneous phenotypic reactions with some plants from a barley line resistant and the others from the same line susceptible. The six homogeneous resistant and four homogeneous susceptible barley lines were tested again against the Indiana and Oklahoma populations (see Materials and Methods), and similar results were obtained (data not shown).

Four rice varieties, 'Azucena' (Japonica), 'Kitaake' (Japonica), 'Nipponbare' (Japonica), and 'IR64' (Indica), were tested against the three different Hessian fly populations described in Materials and Methods. All the four varieties were resistant to the three Hessian fly populations.

Hessian Fly Larvae in Wheat, Barley, and Rice Seedlings. In resistant wheat seedlings, the majority of Hessian fly larvae died within the first 4 d, and no larvae survived >7 d (Fig. 3). In susceptible wheat seedlings, <10% of Hessian fly larvae died within the first 7 d, and <15% died during the entire larval stage. The death curves of Hessian fly larvae in resistant and susceptible barley seedlings were strikingly different from those observed in resistant and susceptible wheat seedlings. In resistant barley seedlings, the death rate

Table 2. Effect of Hessian fly infestation on barley plant growth

Germplasm	Country	Plantid	Not stunted	Stunted	% not stunted
CIho 15478	USA	KLAGES	8	0	100
CIho 15768	USA	PARK	12	0	100
CIho 5041	Germany	Bavarian Brewing Barley	14	0	100
CIho 6804	USA	Wheat Barley	20	0	100
PI 10585	Sweden	HANNCHEN	14	0	100
PI 552963	Canada	HEARTLAND	20	0	100
PI 539132	Germany	PIROLINE	17	1	94
CIho 15553	USA	CM 72	16	1	94
PI 607936	USA	ORCA	15	1	94
CIho 15487	USA	KARL	12	1	92
CIho 6778	USA	Ten Rowed Barley	12	1	92
PI 566953	USA	BRACKEN	11	1	92
PI 63926	China	Rice Barley	20	2	91
CIho 9538	USA	TRAILL	18	2	90
CIho 6129	USA	Black Barley	8	1	89
PI 5873	Germany	Middle Barley	16	2	89
CIho 10613	China	Weed Barley	16	2	89
PI 539106	Ethiopia	ABYSSINIAN	6	1	86
CIho 15687	USA	KIMBERLY	11	2	85
CIho 15865	USA	AZURE	16	3	84
CIho 10858	France	Barley 35/7	10	2	83
CIho 15694	USA	KOMBAR	15	3	83
CIho 5042	Germany	Bavarian Brewing Barley	18	4	82
PI 283391	Hungary	Summer Barley	18	4	82
CIho 10612	China	Weed Barley	13	3	81
CIho 15264	Canada	KLONDIKE	13	3	81
CIho 15514	Canada	HECTOR	13	3	81
CIho 15860	USA	KARLA	12	3	80
PI 562644	USA	OTAL	8	2	80
PI 328052	USA	BARLEY MINNESOTA 331	15	4	79
PI 483238	USA	HAZEN	11	3	79
PI 39400	Australia	Barley No. 36	14	4	78
CIho 10421	USA	UNITAN	14	4	78
CIho 7534	USA	ARIVAT	13	4	76
PI 283392	Hungary	Brewers Barley	14	5	74
PI 542708	Egypt	Egyptian Dryland Barley	11	4	74
CIho 15480	USA	BEACON	8	3	73
CIho 14003	Canada	BONANZA	16	6	73
PI 537576	USA	UC 603	12	5	71
PI 542707	Egypt	Egyptian Dryland Barley	10	4	71
PI 537442	USA	TREBI	7	3	70
PI 605472	USA	GARNET	7	3	70
CIho 10611	China	Weed Barley	11	5	69
CIho 10648	USA	LARKER	8	4	67
CIho 10968	USA	DICKSON	8	4	67
CIho 13796	Canada	PRIMUS II	10	5	67
CIho 9530	USA	HILAND	8	4	67
PI 476976	USA	ROBUST	10	5	67
PI 39398	Australia	Barley No. 22	10	6	63
CIho 15475	USA	SUTTER	13	8	62
PI 592798	Canada	JACKSON	9	6	60
CIho 7248	USA	BONNEVILLE	7	5	58
CIho 4982	Norway	MACHINE BARLEY	8	6	57
PI 63924	China	Awn Barley	8	6	57
CIho 15216	USA	NORDIC	6	5	55
PI 39399	Australia	Barley No. 24	11	9	55
PI 446894	China	Hexagonal White Barley	6	5	55
PI 483237	USA	BOWMAN	6	5	55
CIho 10647	USA	TROPHY	7	6	54
PI 592799	Canada	LEDUC	8	7	53
CIho 6015	Australia	Barley 305	8	8	50
CIho 6969	USA	KINDRED	6	6	50
PI 327889	Pakistan	Barley 5681	6	6	50
PI 361677	Denmark	Barley 16	9	9	50
PI 539105	USA	BENTON	4	4	50
PI 592796	USA	AC ALBRIGHT	6	7	46
PI 564592	USA	MULLIGAN	6	7	46
PI 48093	S. Africa	Barley Wheat	10	12	45
CIho 2376	Ethiopia	ABYSSINIAN	4	5	44
PI 539129	USA	DAYTON	4	5	44
CIho 14119	Ethiopia	Abate	5	7	42

Table 2. (Continued)

Germplasm	Country	Plantid	Not stunted	Stunted	% not stunted
GSHO 956	?	Golden Seeded mother line	8	12	40
CIho 11868	USA	VANGUARD	8	12	40
CIho 15769	USA	GLENN	4	8	33
CIho 15773	USA	MOREX	3	6	33
PI 533600	USA	HAYBET	5	11	31
CIho 15559	USA	BOYER	3	7	30
CIho 13682	USA	BRIGGS	5	12	29
CIho 15774	Canada	BEDFORD	4	10	29
CIho 11638	Canada	CONQUEST	4	11	27
CIho 13826	USA	ERBET	4	12	25
CIho 15766	USA	KOMBYNE	2	6	25
PI 39397	Australia	Barley No. 18	3	10	23
CIho 15857	USA	CLARK	4	14	22
PI 496400	USA	COUGBAR	4	14	22
CIho 13109	USA	PRIMUS	3	12	20
PI 539108	USA	ATLAS	3	12	20
CIho 15856	USA	LEWIS	2	12	14
CIho 7250	USA	PLAINS	4	24	14
CIho 15850	Canada	JOHNSTON	1	11	8
CIho 12119	Denmark	Barley 16	0	13	0
CIho 15229	USA	STEPTOE	0	12	0
CIho 15549	USA	MANKER	0	11	0
GSHO 934	?	Golden Seeded Dwarf	0	22	0

Plantid, plant identification markers.

reached only 40% on the fourth day, and the overall death rate for the whole larval stage reached only 75%. The other 25% of Hessian fly larvae developed successfully into pupae in resistant barley seedlings. In susceptible barley seedlings, the death rate was substantially higher than that in susceptible wheat seedlings. The death rate reached 25% on the fourth day, and the overall death rate for the whole larval stage was 60%. Only 40% of larvae developed successfully into pupae in susceptible barley seedlings. The death rate of Hessian fly larvae in rice seedlings was 40% on the fourth day, similar to the death rate observed in resistant barley, but much lower than that observed in resistant wheat. However, the overall death for the whole larval stage reached 100%, the same as in resistant wheat seedlings.

The growth curves of Hessian fly larvae were also quite different in wheat, barley, and rice seedlings (Fig. 4). In susceptible wheat, Hessian fly larvae grew slowly in the first four days. The growth accelerated from the fifth day (the beginning of the second instar) and reached a plateau on the 11th day (the beginning of the prepupal stage). In susceptible barley seedlings, the growth curve of Hessian fly larvae was similar to that in susceptible wheat seedlings in the first 4 d. However, larval growth slowed down after that, and it took 13 d to reach the prepupal plateau. In resistant barley, growth was slower from the beginning, and it took 15 d for Hessian fly larvae to reach the prepupal plateau stage. In addition to slower growth, the size of the prepupae was also significantly smaller in susceptible barley seedlings and even smaller in resistant barley seedlings compared with those in susceptible wheat seedlings. In rice seedlings, Hessian fly larvae grew a little in the first 6 d, but no larva transformed into second instars. In resistant wheat seedlings, no growth in larvae was observed before death.

Emergence Rates of Hessian Fly Pupae in Wheat and Barley Seedlings. Despite the differences in larval death rate, larval growth rate, and prepupal size, adult emergence rates from pupae in susceptible wheat, susceptible barley, and resistant barley seedlings were very similar. The emergence rates of pupae obtained from susceptible wheat, susceptible barley, and resistant barley seedlings were not statistically different ($\chi^2 = 5.495$, df $= 2$, $P = 0.064$) (Fig. 5).

Discussion

Host Preference and Tissue Discrimination. Plant-feeding gall midges generally have very narrow host ranges (Harris et al. 2003). For example, hosts of *Orseolia oryzae* L. include primarily rice and wild rice species. For the Hessian fly, wheat is the preferred host. Barley, rye, triticale, and wild grasses, including *A. repens, A. smithii, E. virginicus, E. canadensis,* and *Aegilops* sp., are suspected to be able to serve as alternative hosts when wheat is not available (Jones 1936, 1938, 1939; Harris et al. 2001). The alternative hosts are thought to be critical in the preservation of Hessian fly populations in the wild when wheat plants are not available. However, there has been no convincing evidence that the Hessian fly can reproduce in wild grasses (Zeiss et al. 1993). The impact of the Hessian fly on the yield of the alternative hosts except barley has not been evaluated either. Jones (1938) observed that some alternative hosts exhibited no injury from Hessian fly attacks, whereas other alternative hosts exhibited good recovery from the initial injury. These observations indicate that Hessian fly infestation might cause little yield loss to some of the alternative host species.

Wild barley species have been reported to be alternative hosts for the Hessian fly in nature as well as
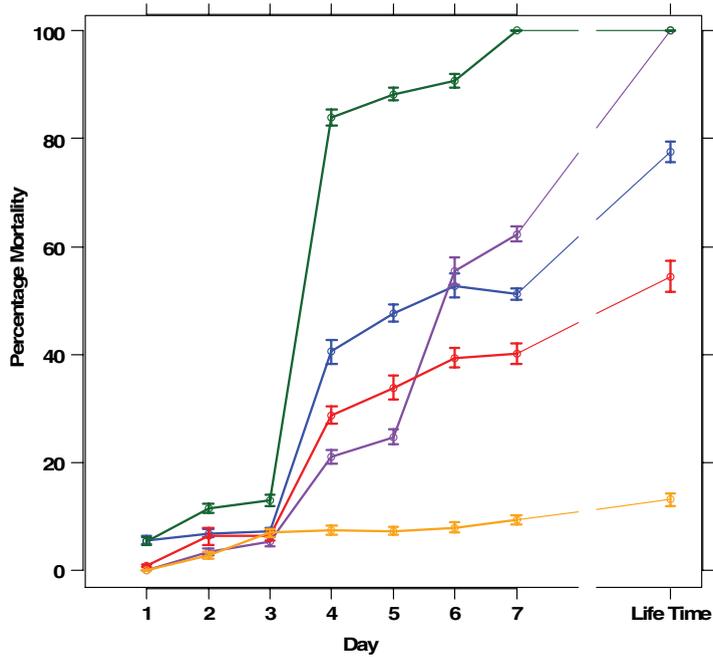
Fig. 3. Death curves of Hessian fly larvae in wheat, barley, and rice seedlings. The orange, red, blue, pink, and green lines present susceptible wheat (Newton), susceptible barley (CIho 15229), resistant barley (CIho 15768), rice (Nipponbare), and resistant wheat (Molly), respectively.

under artificial conditions (Jones 1936). In the west coast region of the United States, the Hessian fly is suspected to cause damage to barley. Consequently,

barley breeders are breeding for Hessian fly resistance in barley cultivars. Barley breeding lines are also being tested against Hessian flies collected from wheat fields
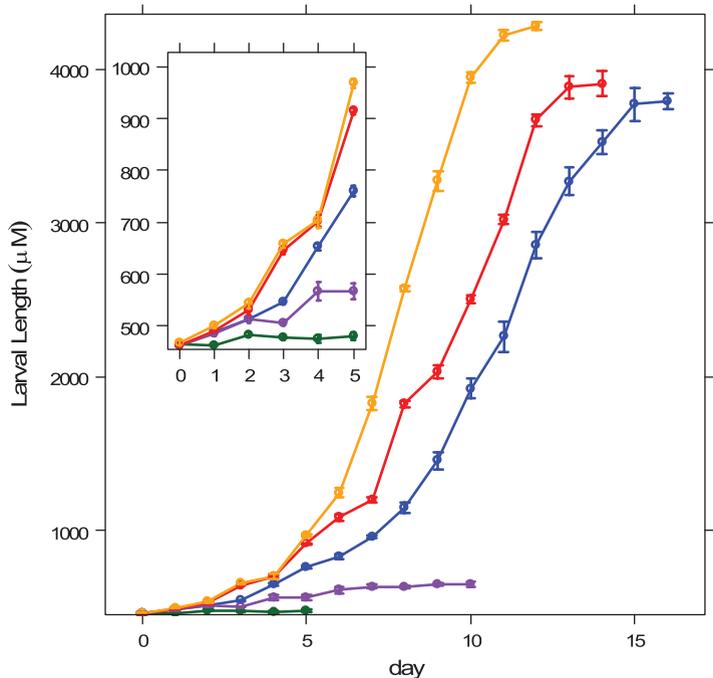
Fig. 4. Growth curves of Hessian fly larvae in wheat, barley, and rice seedlings. The inserted figure represents the data of the first 5 d. The orange, red, blue, pink, and green lines present susceptible wheat (Newton), susceptible barley (CIho 15229), resistant barley (CIho 15768), rice (Nipponbare), and resistant wheat (Molly), respectively.
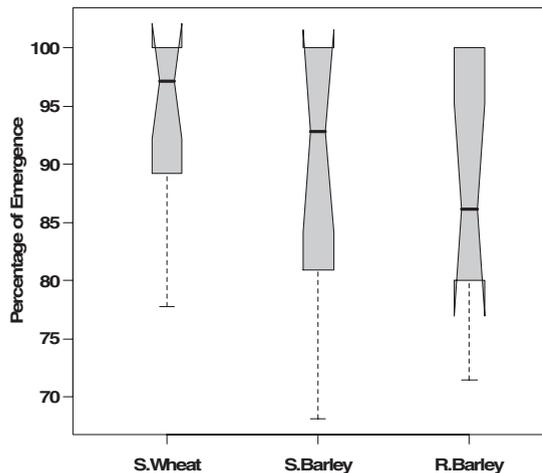
Fig. 5. Emergence rates of Hessian fly pupae in different plant types. A notched box contains the middle 50% of a data group; the bold line inside a box represents the median; and the two horizontal lines linked to a box by a vertical, dashed line represent the upper and lower 25% of the data. Overlapped notches indicate no difference in the median percentages of data groups (Chambers et al. 1983). *P* values are 0.136 between S wheat and S barley, 0.065 between S wheat and R barley, and 0.363 between S barley and R barley.

for seedling resistance. According to our observations and previous reports (Jones 1936, Harris et al. 2001), barley seedlings were much less attractive to adults for egg deposition when barley and wheat were grown next to each other. Hessian fly adults deposited three times more eggs on wheat seedlings than on barley seedlings. In addition, Hessian fly adults discriminated poorly between the adaxial leaf surface, abaxial leaf surface, and C&FLS on a barley seedling, and deposited almost half of their eggs (49.4%) on the abaxial leaf surface and C&FLS, where newly hatched larvae will die due to unable entrance into the interspace between leaf sheaths. This was in contrast to the fact that <14% eggs were found on the abaxial leaf surface and C&FLS on a wheat seedling. Similar differences in egg distributions between wheat and barley seedlings were also observed by Kanno and Harris (2000a,b). Assuming a similar situation in the field, a much larger Hessian fly population will be needed to cause similar damage to barley than that needed to wheat. Moreover, Hessian fly larvae did poorly in barley seedlings compared with Hessian fly larvae in wheat seedlings (see below).

The inability of Hessian fly adults to discriminate the adaxial leaf surface from the abaxial leaf surface and C&FLS on a barley seedling was probably due to the shape and position of a barley leaf. A barley leaf is much bigger than a wheat leaf. Because its bigger size, a barley leaf often lays flattened or even inverted, whereas a wheat leaf is upright. Ovipositing Hessian fly adults prefer upright over flattened or inverted leaves (Wendell 1981). Consistent with this postulation, Hessian fly adults discriminate the adaxial surface from the abaxial surface of a leaf very well on a rice

seedling, which is nonhost. Like a wheat leaf, a rice leaf at the seedling stage is small and holds upright.

Barley–Hessian Fly Interaction. Even though Hessian fly larvae can complete their life cycle in barley seedlings, the barley–Hessian fly interaction seemed different from the wheat–Hessian fly interaction. A high percentage of barley lines exhibited Hessian fly resistance. Of the 94 lines screened, 6.4% of the lines exhibited homogeneous resistance to the Hessian fly, 89.3% of the lines exhibited partial resistance, and only 4.3% of the lines were homogeneous susceptible (Table 1). This is in contrast to a low percentage of wheat germplasm lines that are Hessian fly resistant. One needs to screen a large number of wheat genotypes to identify a line that is Hessian fly resistant (El Bouhssini et al. 1999; M.-S.C. et al., unpublished observation).

The death rate ($\approx$60%) of Hessian fly larvae in susceptible barley seedlings was dramatically higher than that ($\approx$10%) of Hessian fly larvae in susceptible wheat seedlings (Fig. 3). Hessian fly larvae in susceptible barley also grew more slowly than those in susceptible wheat (Fig. 4).

The resistance mechanism observed in the examined barley lines was also different from that mediated by major known *R* genes in wheat. In wheat, the resistance conferred by major existing *R* genes is antibiosis (Hatchett and Gallun 1970). Hessian fly larvae die in resistant wheat seedlings within 4 to 7 d without growth and development (Fig. 2). In the examined resistant barley lines, the resistance mechanism seemed to be a combination of antibiosis and tolerance. The evidence for antibiosis in the resistant barley lines came from the overall high death rate (>75%). A large proportion ($\approx$40%) of Hessian fly died at the fourth day without growth and development. The evidence for tolerance came from the fact that a small percentage ($\approx$25%) of Hessian fly larvae could survive in resistant barley seedlings and were able to develop into pupae, even though the sizes of the pupae in resistant barley seedlings were much smaller than those in susceptible wheat seedlings (Fig. 4). However, Hessian fly larvae in resistant barley seedlings did not inhibit the growth of the plants significantly (Fig. 2) and did not affect the tillering ability of a barley seedling either (data not shown).

The screening of resistant and susceptible barley lines listed in Table 2 was based on the phenotypes of barley seedlings 16 d after the initial Hessian fly attack. The growth of the barley seedlings inhibited at that time was taken as susceptible plants, whereas the barley seedlings that grew relatively normally were taken as resistant plants. This method can define resistant and susceptible wheat seedlings correctly because the growth inhibition of susceptible wheat seedlings is irreversible and the inhibited plants will die eventually. The situation for barley, however, was different. The inhibition of plant growth at an earlier stage could be restored at a later stage for some of the susceptible barley plants. Similar phenomena also were observed with other Hessian fly alternative hosts (Jones 1938). As shown in Figs. 3 and 4, the distinction between the resistant and susceptible barley lines was not as dra-

matic as the distinction between the *R*-gene–mediated resistant and susceptible wheat lines.

Rice–Hessian Fly Interaction. The rice–Hessian fly interaction was different from both the barley–Hessian fly and wheat–Hessian fly interactions. Tests of four rice varieties against three different Hessian fly populations (Chen et al. 2009) that contain many different biotypes resulted in similar phenotypes for both the host plants and the attacking Hessian fly larvae (data not shown). For the host plants, some initial growth inhibition was observed compared with unattacked plants. Growth recovered later soon after the death of the attacking Hessian fly larvae. The initial growth inhibition of the attacked rice seedlings might reflect the chemical cost associated with host defense responses. The tillering ability of the attacked rice seedlings was not apparently affected (data not shown). For the attacking insects, 100% of Hessian fly larvae died eventually in rice seedlings, as was the case in resistant wheat (Fig. 3). However, the death mode of Hessian fly larvae in rice seedlings was different from that of Hessian fly larvae in resistant wheat. In rice, Hessian fly larvae died gradually, and a small percentage of the larvae survived as long as 12 d. In comparison, >80% of Hessian fly larvae died within the first 4 d, and no larvae survived >7 d in resistant wheat seedlings. In addition, Hessian fly larvae in rice seedlings grew a little, whereas those in resistant wheat seedlings did not grow at all. The different death modes of Hessian fly larvae in rice and resistant wheat seedlings indicated different resistance mechanisms between nonhost rice and wheat containing major *R* genes.

Another interesting characteristic of the rice–Hessian fly interaction was the very low percentage (11%) of the larvae that migrated successfully into the interspaces between leaf sheaths. The majority of larvae was unable to enter rice seedlings and died on the leaf surface. This phenomenon indicated that some characteristics of rice leaves might have prevented Hessian fly larvae from finding the right path to the entrance of the interspace between leaf sheaths.

In conclusion, barley is a host, but not the best host for the Hessian fly. The poor discrimination of adult females in selecting the adaxial leaf surface from abaxial leaf surface and C&FLS of a barley seedling during oviposition results in fewer viable offspring larvae. The high death rate and slow development of Hessian fly larvae in barley seedlings suggest poor population growth. In addition, barley seedlings exhibited strong tolerance and good recovery from Hessian fly attacks. These observations suggest that Hessian fly should not be a major problem for barley. However, crop damages and yield losses have been observed in barley fields (Buntin and Raymer 1992). Further research is needed to clarify this dilemma. For example, Hessian fly larvae may not affect barley seedlings seriously at temperature at 20°C or below but may become more damaging at higher temperature (Olembo et al. 1966). The impact of Hessian fly larvae on adult barley plants also needs to be examined. In addition, damages to barley also could be caused by a

different species or subspecies. In Morocco, two sympatric species of *Mayetiola* (*destructor* and *hordei*) have been identified on the basis of microscopic characters (Gagné et al. 1991). Barley is almost exclusively colonized by *M. hordei*, whereas wheat is almost exclusively colonized by *M. destructor.* Two different forms of *M. hordei* exist, one form is able to produce distinct stem swellings (galls), whereas the other form does not produce apparent galls (Makni et al. 2000). If the insect that causes damage to barley is also a different race or species in the United States, we will need to reconsider our strategy for controlling barley damage. The current practice of breeding barley cultivars with resistance to Hessian flies collected from wheat fields needs to be reevaluated.

The resistance mechanisms associated with wheat cultivars containing major *R* genes, resistant barley lines examined in this study, and nonhost rice seemed different. Resistance in wheat conferred by major *R* genes kills Hessian fly larvae via an acute antibiosis that is activated by a gene-for-gene interaction (Hatchett and Gallun 1970). Resistance in the examined barley lines is likely a combination of antibiosis and tolerance. Rice is a nonhost for Hessian fly. The fact that Hessian fly larvae live longer and grow a little in rice seedlings in comparison with those in resistant wheat indicates distinct resistance mechanisms between rice and wheat. Our microarray analyses of gene expression in rice and wheat seedlings attacked by Hessian fly larvae also indicated the activation of different defense pathways between rice and wheat (M.-S.C. et al., unpublished data). Elucidation of these different resistance mechanisms may lead to new strategies for the development of plants with more durable resistance.

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