α-Amylase Inhibitors from Wheat Kernels as Factors in Resistance to Postharvest Insects

M. A. YETTER[1], R. M. SAUNDERS[1], and H. P. BOLES[2]

ABSTRACT

α-amylase inhibitors were extracted from kernels of five hard winter wheat varieties that were grown at different locations in two crop years and that had been evaluated for susceptibility or resistance to the rice weevil, *Sitophilus oryzae* (L.). Inhibitor activity was assayed with larval α-amylase from two species of stored grain pests, the rice weevil and the yellow mealworm, *Tenebrio molitor* L. Correlation in some wheat varieties was observed between in vivo resistance to the insect and the extent of in vitro inhibition of the insect larval α-amylase by the extracted inhibitors. These results indicate that α-amylase inhibitors in wheat could be involved in the resistance of wheat to postharvest infestation.

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Although the isolation and general properties of α-amylase inhibitors in wheat and other cereal grains have been described by many workers (Kneen and Sandstedt 1943, 1946; Militzer et al 1946a,b; Saunders and Lang 1973; Silano et al 1973), the physiological function of these proteinaceous inhibitors has not been determined. The inhibitors are active against insect and mammalian amylases but not plant amylases. This phenomenon suggests that the inhibitors do not serve as regulatory agents in wheat kernel α-amylase metabolism (Kneen and Sandstedt 1943, Marshall and Lauda 1975, Shainkin and Birk 1970, Silano et al 1973).

One function proposed in the literature (Marshall and Lauda 1975, Silano et al 1975) is that these inhibitors could act as a natural defense mechanism against predatory insects by rendering inactive the digestive α-amylase enzyme of the attacking insects. Applebaum (1964) and co-workers (1964, 1965) showed that wheat inhibitors were active against insect amylases both in vitro and in vivo. Silano et al (1975) speculated that inhibitors exist in wheat as naturally occurring insect resistance factors when they reported that insect species normally attacking wheat grains and wheat products had higher amylase activities and were more susceptible to inhibition by wheat inhibitors than were the amylases of those insects that did not attack wheat.

If amylase inhibitor activity in wheat could be shown to be directly related to resistance to insect pests, it could serve as an important index for selecting and genetically breeding improved wheat varieties, and also could provide breeders with a rapid, easy test for predicting resistance to insects. In turn, the development of more insect-resistant wheat varieties would have an important effect on the world food supply by reducing the volume of stored wheat and wheat products lost each year to insect infestation.

This paper correlates α-amylase inhibitor activity in wheat with postharvest insect resistance.

MATERIALS AND METHODS

Test Grains.

Four of the wheat varieties used in this study are of recent release: Satanta (1969), Eagle (1970), Centurk (1971), and Trison (1973). The fifth variety, Turkey, originated in the Crimea and was brought to the United States in 1873.

Samples of the 5 varieties, grown at 9 different Kansas Experiment Station sites in two crop years, 50 cultivars in total, were sent to the U.S. Grain Marketing Research Laboratory immediately after harvest. The plants of each variety from which the grains were harvested in each crop year were grown from the same lots of seed stock. The grains were stored in insect-free containers up to the time of testing. Moisture was equilibrated by placing the test samples in a room controlled at 26 ± 1°C and 60 ± 2% RH for four weeks.

Test Insects.

The 14-day ± 7-day-old *Sitophilus oryzae* (L.) were from the standard Manhattan laboratory strain maintained on Scout variety wheat. The adults were sieved out just prior to testing.

Test Procedures.

Six female and three male weevils were caged on 100-kernel samples from each of the 50 wheat cultivars for a seven-day oviposition period. The weevils were then removed, and the grain samples held in a rearing room until the adult progeny emerged. Adult progeny were removed from the samples each day until the 60th day after the start of oviposition. The mean progeny number from five test replications was used as a measure of the susceptibility or resistance of the grains to rice weevil infestation and development.

Isolation of Protein Inhibitor.

A crude inhibitor mixture was prepared from each of the wheat samples grown at the different sites in the two crop years. Five hundred milligrams of finely ground wheat kernels was gently shaken in 30 ml of 0.1*M* sodium bicarbonate solution, pH 8, for 10 min. The soluble extract was separated from insoluble residue by centrifugation for 20 min at 7,700 × *g*. The supernatant soultion was heated at 70°C for 10 min (no inhibitor loss occurs under these conditions), dialyzed against the sodium bicarbonate solution overnight, and frozen until assayed.

Assay of Inhibitor Activity.

Assays were run against α-amylase from the larvae of *S. oryzae*, and the yellow mealworm, *Tenebrio molitor* L. Each assay was repeated a minimum of four times to obtain a reliable estimate of the mean. The larval enzyme extracts were prepared according to Applebaum et al (1961) using 0.03*M* acetate barbiturate buffer, pH 5.4, containing 0.44*M* sodium chloride and 0.001*M* calcium chloride, and assayed in the same buffer.

Inhibitor activity in each of the 50 wheat samples was measured by adapting the method of Bernfeld (1965) in the following way: Appropriate amounts of inhibitor solution (ie, wheat extract) of 0 to 50 μl were incubated for 30 min in 1.9 ml of buffer and 100 μl of α-amylase diluted to a standard activity. Starch (1 ml of a 1% solution in water) was then added, and the reaction was allowed to proceed for 10 min, after which it was stopped by the addition of dinitrosalicylic acid reagent.

With appropriate control experiments, the absorbance values at 530 nm were used to calculate the extent of inhibition:

[1]Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Albany, CA 94710.
[2]Grain Marketing Research Center, Manhattan, KS 66506.

$$\% \text{ Inhibition for an aliquot} = \frac{\text{Absorbance, control (no inhibitor)} - \text{absorbance, sample}}{\text{Absorbance, control (no inhibitor)}} \times 100$$

Conditions were deliberately chosen so that inhibition was measured only on the linear portion of the inhibitor-substrate reaction curve to allow simple quantitative comparison among the different substrates (Saunders 1975).

RESULTS AND DISCUSSION

Inhibitor extracts from all 50 wheat samples were active against the amylases from *S. oryzae* and *T. molitor* larvae. Since space does not permit reporting the data for all cultivars studied, a representative portion of the data collected is shown in Table I. The values are those obtained from the 10 cultivars of the Centurk variety. Listed are growth location, crop year, progeny number (the measurement of in vivo insect resistance), and percent amylase inhibited by the extracted inhibitors. Each variety was grown in both the 1973 and 1974 crop years; however, growth locations were not identical from year to year or among varieties.

Correlation coefficients, r, between progeny number and percent α-amylase inhibition for all 50 cultivars and for each variety are listed in Table II. A test of significance was performed for a negative r value, the sign of interest in relating α-amylase inhibition to progeny development. Probability levels at which r is judged significant are given in parentheses when below 10%. Overall correlations were significant at the 7 and 3% levels for *T. molitor* and *S. oryzae*, respectively.

Centurk and Eagle varieties had r values significant near the 1% level for progeny number correlated with *S. oryzae* amylase inhibition. Correlations with *T. molitor*, although lower, were significant for two varieties, Eagle and Satanta, at approximately the 5% level. Although other work (Silano et al 1975) has shown *T. molitor* and *S. oryzae* to have similar α-amylase inhibitor characteristics, correlations with progeny emergence data obtained from *S. oryzae* would not be expected to be as high for *T. molitor* as for *S. oryzae*. The range of values found for progeny number and α-amylase (*S. oryzae*) inhibitor levels in the five varieties were as follows: Trison, 19–40, 68–77%; Centurk, 24–38, 55–71%; Eagle, 17–45, 60–71%; Satanta, 15–44, 63–78%; Turkey, 27–40, 47–73%. These data do not indicate a threshold inhibitor value for progeny number effects.

The significance of correlations reported in this paper lends support to the hypothesis of a relation between insect progeny development and α-amylase inhibitor activity. Considering the limited origins of wheat samples available for this study, the evidence is encouraging that inhibitor levels may be a valid measure of resistance of wheat to postharvest insects. Only two crop years of wheat grown at locations within one state were tested. This makes it difficult to determine what effect environment may have had on the values of progeny number and inhibitor activity. A more extensive study covering several years and growing regions, although difficult to execute, could reveal more valid correlation data. The information presented in this paper should stimulate a more comprehensive study of the subject.

TABLE I
Data Collected for the 10 Cultivars of Centurk

Growth Location	Crop Year	*Sitophilus Oryzae* Progeny Number	% Inhibition of Larval α-Amylase from *Tenebrio molitor*	% Inhibition of Larval α-Amylase from *S. oryzae*
St. John	1973	24.0	55.7	66.3
Colby	1973	30.4	66.2	55.4
Hutchinson	1973	32.0	62.0	60.1
Minneola	1973	37.6	57.6	54.9
Tribune	1973	29.0	63.1	66.7
Colby	1974	35.2	67.1	61.3
Hutchinson	1974	26.2	60.7	66.5
Manhattan	1974	29.4	67.4	66.2
Minneola	1974	24.0	69.0	70.9
Tribune	1974	29.6	52.4	54.5

TABLE II
Correlation Coefficients, r, between *Sitophilus oryzae* Progency Number and Percent Inhibition of *Tenebrio molitor* and *S. oryzae* α-Amylase

Wheat Variety	r values (p)[a] *T. molitor*	r values (p)[a] *S. oryzae*
Trison	−0.138	0.178
Centurk	−0.213	−0.699 (1.3%)
Eagle	−0.580 (4.0%)	−0.723 (0.9%)
Satanta	−0.538 (5.5%)	−0.301
Turkey	0.142	0.064
All varieties	−0.210 (7.2%)	−0.267 (3.1%)

[a] Probability level at which r is significant.

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