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# Susceptibility of stored-product beetles on wheat and maize treated with thiamethoxam: effects of concentration, exposure interval, and temperature☆

Frank H. Arthur[a,*], Bisong Yue[b], Gerald E. Wilde[c]

[a] *Grain Marketing and Production Research Center, USDA-ARS, Manhattan, KS 66502, USA*
[b] *Department of Biology, Sichuan University, Chengdu, Sichuan, People's Republic of China*
[c] *Department of Entomology, Kansas State University, Manhattan, KS, USA*

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**Abstract**

*Sitophilus zeamais* Motschulsky, the maize weevil, *Oryzaephilus surinamensis* (L.), the saw-toothed grain beetle, and *Tribolium castaneum* (Herbst), the red flour beetle, were exposed for 1, 2, 3, and 6 d at 22°C, 27°C, and 32°C on maize treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam, a new-generation neonicotenoid insecticide. A second series of tests was conducted on hard winter wheat using *S. oryzae* (L.), the rice weevil, *Rhyzopertha dominica* (F.), the lesser grain borer, and *T. castaneum*. Mortality of all species on both commodities generally increased with insecticide concentration, exposure interval, and temperature, and data were described by linear and non-linear regressions with concentration as the independent variable. Mortality of *S. zeamais* ranged from 58% to 90% on maize treated with 0.5 ppm thiamethoxam, and approached 95–100% as concentration increased to 4 ppm. *Oryzaephilus surinamensis* appeared to be slightly less susceptible than *S. zeamais*; mortality ranged from about 18% to 80% at 5 ppm and there was a more gradual increase in mortality as concentration increased. Mortality of *T. castaneum* generally did not exceed 40% at any concentration unless the beetles were exposed for 6 d. Mortality of *R. dominica* and *S. oryzae* was less than 60% when exposed on treated wheat for 1 and 2 d, but increased to nearly 100% when exposed for 6 d at 27°C and 32°C. Mortality of *T. castaneum* did not exceed 20% at the 1- and 2-d exposures, and approached 100% only when beetles were exposed for 6 d at 32°C. Few $F_1$ adults of any species were found in treated maize or in treated wheat but the number of $F_1$ *T. castaneum* in

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*Corresponding author. Tel.: +1-785-776-2783; fax: +1-785-537-5584.
*E-mail address:* arthur@gmprc.ksu.edu (F.H. Arthur).

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untreated maize and untreated wheat was very low compared with the other species. Results show that thiamethoxam would be an effective protectant of stored maize seed and stored wheat seed.


*Keywords:* Beetles; Stored wheat; Stored maize; Thiamethoxam

## 1. Introduction

Thiamethoxam is a broad spectrum neonicotenoid contact insecticide developed and manufactured by Syngenta (formerly Novartis Crop Protection). Neonicotinoids interfere with the nicotinic acetylcholine receptor and therefore have specific activity against the insect nervous system (Hofer and Brandl, 1999; Maienfisch et al., 2001). This unique mode of action makes them highly desirable for controlling insects that are developing resistance to conventional organophosphate, carbamate, and pyrethroid insecticides (Maienfisch et al., 1999).

Thiamethoxam also has minimal effects on beneficial insects, low toxicity toward mammals, and does not produce teratogenic or mutagenic effects (Lawson et al., 1999). Because of this selective activity, thiamethoxam has been evaluated as a seed treatment for several major field crops, including cotton (Hofer and Brandl, 1999). Low application rates controlled early season piercing-sucking insects and soil insects (Hofer and Brandl, 1999; Zang et al., 1998, 1999). In addition, in some field crops thiamethoxam is systemic and can be transported to untreated areas of the plant (Lawson et al., 1999). Thiamethoxam is also effective at low rates against termites (Delgarde and Rouland-Lefevre, 2002), and against blueberry maggot flies exposed on spheres coated with thiamethoxam (Ayyappath et al., 2000).

There are no published studies in which thiamethoxam has been evaluated for control of stored-product beetles. The objectives of this study were to: (1) determine efficacy of thiamethoxam for controlling stored-product beetles on seed maize and seed wheat; (2) evaluate several different exposure intervals for insects and examine the interaction between application rate and exposure interval; (3) determine suppression of the $F_1$ generation when adults are exposed; and (4) evaluate effects of temperature on insect mortality. Bioassays on maize were conducted with *Sitophilus zeamais* Motschulsky, the maize weevil, *Tribolium castaneum* (Herbst), the red flour beetle, and *Oryzaephilus surinamensis* (L.), the saw-toothed grain beetle. Bioassays on wheat were conducted with *Rhyzopertha dominica* (F.), the lesser grain borer, *S. oryzae* (L.), the rice weevil, and *T. castaneum*. These species were selected because *R. dominica*, *S. oryzae*, and *S. zeamais* are important internal feeders and produce insect damaged kernels (IDK), while *T. castaneum* and *O. surinamensis* are common external pests on stored grains throughout much of the world.

## 2. Materials and methods

### 2.1. Experiment 1: evaluation as a protectant of maize seed

The formulation of thiamethoxam (Cruiser®) used in this study was a 600 mg/ml (5 lb/gal) active ingredient (AI) emulsifiable concentrate (EC) supplied by Syngenta, Inc. (Greensboro, NC, USA). Application rates evaluated were 0 (untreated control), 0.5, 1.0, 2.0, and 4.0 ppm, and these specific rates were chosen as a comparison to the current label rates of 6 ppm for chlorpyrifos-methyl on wheat and 6–8 ppm for pirimiphos-methyl on maize. Both of these insecticides are neurotoxic organophosphate compounds. Also, preliminary tests had shown that a 1-week exposure interval on maize treated with 4 ppm thiamethoxam killed all *S. zeamais* adults. Three insect species, *S. zeamais*, *O. surinamensis*, and *T. castaneum*, were used for bioassays, and the insecticide evaluations were conducted at 22°C, 27°C, and 32°C. The maize was a mixture of several unknown varieties obtained from local growers. Individual bioassays consisted of 25 g maize held inside 40 ml plastic vials, which were capped with a screened lid. Beetles were exposed for 1, 2, 3, and 6 d, and mortality was determined upon completion of the respective exposure intervals. There were six replicates for each treatment, and because of the large numbers of insects required for the bioassays and the complexity of the test, each replicate was done separately at 2-week intervals.

Treatment procedures for an individual replicate were as follows. Three humidity chambers, one for each temperature, were created inside $26 \times 36.5 \times 15\,cm^3$ plastic boxes with waffle-grid cut to fit the bottoms, and approximately 750 ml of saturated NaBr was poured into the bottom of each box to maintain relative humidity (r.h.) of 57–60%, which approximates to 12.5% moisture content in grains (Greenspan, 1977). Thirty-six vials (three temperatures × four exposure intervals × three species) and 900 g of maize were needed for each of the five concentrations (including the untreated control). However, 1.5 kg of maize was treated to ensure complete coverage. Each lot of 1.5 kg consisted of 10% cracked and broken maize. The normal volume spray rate for thiamethoxam when used as a seed treatment is 6.5 ml of formulated spray/kg (10 oz/100 lb) or 9.8 ml/1.5 kg.

Each treatment concentration was formulated by making serial dilutions to produce stock solutions that would give 0.5, 1.0, 2.0, and 4.0 ppm when sprayed at the rate of 9.8 ml/1.5 kg of maize. The control treatment was applied by using a Badger 100 artists' airbrush (Franklin Park, IL, USA) to spray 9.8 ml of tap water onto the maize, which was spread out on a $0.6 \times 0.3\,m^2$ flat plywood surface. Each of 36 vials was then filled with 25 g maize and the remainder of the maize was discarded. In the next step, the vials were divided into three groups of 12. Twenty mixed-sex adult *S. zeamais* were put into each vial in one group, 20 adult mixed-sex *O. surinamensis* were put into each vial in the second group, and 20 adult mixed-sex *T. castaneum* were put into each vial in the third group. All adults were about 1–2-week old, and were obtained from pesticide-susceptible laboratory cultures reared at 60% r.h., 27°C. Four vials from each group (one for each exposure interval of 1, 2, 3, and 6 d) were put into each of the three humidity chambers with the saturated NaBr solution. Each concentration of thiamethoxam was then applied in succession to different lots of 1.5 kg maize using the airbrush to mist the solution onto the maize, and the vials were filled, divided in the same manner as described for the untreated controls, and placed into the humidity chambers. After the treatments were concluded, one chamber was then put inside an incubator set at 22°C, the second inside an incubator set at 27°C, and the third inside an incubator set at 32°C. Temperature and relative humidity inside each box were monitored using HOBO recording computers (Poccassett, MA, USA). The optimum developmental temperatures for most stored-grain beetles, including the species selected for the trials in this study, are approximately 23.9–33.3°C (Howe, 1965; Fields, 1992); therefore, the temperatures reflect the normal developmental range and were appropriate for the study.

Upon completion of each exposure interval, the vials were removed from the temperature incubator and the humidity chambers, the maize was sifted and insect mortality assessed; then the maize was put back into the vials. The vials were in turn returned to the chambers and the temperature incubators and held for an additional 8 weeks; then the maize was sifted to collect $F_1$ adults and discarded.

For each successive replicate, new humidity chambers were created, and the maize was treated and insects exposed and processed in the same manner as described in the preceding paragraphs. Maize was treated sequentially from the lowest to the highest concentration. Data were analyzed separately by species, with treatment, exposure, and temperature as main effects or factors, with mortality after exposure and $F_1$ adults as the variables of interest. Analysis was performed using the ANOVA, GLM, REG, and MEAN procedures of the Statistical Analysis System (SAS Institute, 2001). Significance was determined at the 0.01 level unless noted otherwise. For each temperature–exposure interval combination, Table curve 2D software (Jandel Scientific, 2002, San Rafael, CA, USA) was used to determine the variation explained by any model that could be fitted to the data (maximum $R^2$) and the equation that gave the best fit to the data, based on the amount of variation explained by the chosen equation ($R^2$).

### *2.2. Experiment 2: evaluation as a protectant of hard red winter wheat seed*

Tests were conducted on hard red winter wheat as described for maize, except that the insects used for the bioassays were *R. dominica*, *S. oryzae*, and *T. castaneum*, 30 g of wheat were put into each vial instead of 25 g, and there were five replications instead of six. Each replicate of 1.5 kg wheat consisted of 10% cracked and broken wheat, and all treatments, experimental processes, and methods of exposure and holding the wheat were exactly as described for maize. In addition, new humidity chambers were created for these tests with wheat, as described in the experimental methods for maize. Data collection, statistical analysis, and the curve-fitting process and analysis were also performed in the same manner as described for the tests with maize.

## 3. Results

### *3.1. Experiment 1: evaluation as a protectant of maize seed*

Main effects temperature ($F = 55.2$; df = 2, 300), exposure interval ($F = 10.7$; df = 3, 300), and application rate ($F = 1045.4$; df = 4, 300), and the temperature × rate interaction ($F = 6.2$; df = 8, 300) were significant for mortality of *S. zeamais* exposed on maize treated with thiamethoxam ($P < 0.01$). None of the other interactions were significant: temperature × exposure ($F = 1.2$; df = 6, 300; $P = 0.29$), exposure × rate ($F = 0.9$; df = 12, 300; $P = 0.56$), and temperature × exposure × rate ($F = 0.9$; df = 24, 300; $P = 0.60$). All equations fitted the data extremely well, as shown by the adjusted $R^2$ as a percentage of the maximum that could be fitted to the particular data sets (Table 1). Mortality at 0.5 ppm ranged from 58% to 90%, and also varied among the three temperatures (Fig. 1A–L). At each temperature, mortality increased sharply as concentration went from 0 to 0.5 ppm, and approached a plateau of 95–100% as concentration

Table 1
Parameters (mean ± SE) for non-linear equations $Y = ax^b$ for mortality ($Y$) of *Sitophilus zeamais* exposed for 1, 2, 3, or 6 d at 22°C, 27°C, or 32°C (temperature), on maize treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam ($x$)

| Exposure (d) | Temperature (°C) | $a$ | $b$ | $R^2$ | Max $R^2$ | % of max |
|---|---|---|---|---|---|---|
| 1 | 22 | 67.7 ± 2.6 | 0.104 ± 0.032 | 0.83 | 0.85 | 97.6 |
| | 27 | 77.3 ± 2.6 | 0.100 ± 0.026 | 0.86 | 0.87 | 98.9 |
| | 32 | 86.2 ± 1.5 | 0.101 ± 0.016 | 0.96 | 0.97 | 99.0 |
| 2 | 22 | 74.6 ± 2.5 | 0.105 ± 0.029 | 0.86 | 0.88 | 97.7 |
| | 27 | 77.8 ± 2.1 | 0.145 ± 0.028 | 0.93 | 0.94 | 98.9 |
| | 32 | 88.2 ± 1.8 | 0.087 ± 0.013 | 0.94 | 0.95 | 98.9 |
| 3 | 22 | 74.8 ± 2.2 | 0.096 ± 0.022 | 0.89 | 0.90 | 98.9 |
| | 27 | 79.6 ± 2.4 | 0.155 ± 0.032 | 0.90 | 0.94 | 95.7 |
| | 32 | 88.1 ± 1.1 | 0.091 ± 0.001 | 0.98 | 0.98 | 100 |
| 6 | 22 | 75.8 ± 1.9 | 0.079 ± 0.015 | 0.90 | 0.91 | 98.9 |
| | 27 | 89.9 ± 1.2 | 0.085 ± 0.008 | 0.97 | 0.97 | 100 |
| | 32 | 92.9 ± 1.0 | 0.078 ± 0.006 | 0.98 | 0.99 | 99.0 |

*Note*: Also included are the $R^2$ values of the equations, the maximum (max) $R^2$ that could be obtained from the data set, and the estimated $R^2$ as a percentage of the maximum.

increased to the maximum tested rate of 4 ppm (Fig. 1A–L). Mortality also generally increased as the exposure interval increased.

Main effects temperature ($F = 32.8$; df = 2, 300), exposure interval ($F = 75.8$; df = 3, 300), and application rate ($F = 288.7$; df = 4, 300), plus the temperature × exposure ($F = 3.1$; df = 6, 300), exposure × rate ($F = 4.8$; df = 12, 300), and temperature × rate ($F = 4.0$; df = 8, 300) interactions were all significant for mortality of *O. surinamensis* exposed on maize treated with thiamethoxam ($P < 0.01$). The overall interaction of temperature × exposure × rate was not significant ($F = 0.9$; df = 24, 300; $P = 0.36$). Non-linear equations also fitted the data for each individual combination (Table 2). However, in contrast to the data for *S. zeamais*, mortality of *O. surinamensis* on maize treated with 0.5 ppm thiamethoxam ranged from about 18% to 80%, depending on the exposure interval, and there was a more gradual increase in mortality with increasing concentrations (Fig. 2A–L). Mortality again generally increased with temperature and exposure interval.

Main effects temperature ($F = 51.6$; df = 2, 300), exposure interval ($F = 516.2$; df = 3, 300) and application rate ($F = 249.1$; df = 4, 300), and the temperature × exposure ($F = 5.4$; df = 6, 300), exposure × rate ($F = 32.6$; df = 12, 300), and temperature × rate ($F = 5.5$; df = 8, 300) interactions were all significant for mortality of *T. castaneum* exposed on maize treated with thiamethoxam ($P < 0.01$). The overall temperature × exposure × rate interaction was not significant ($F = 0.9$; df = 24, 300; $P = 0.08$). The values for maximum available $R^2$ values were sometimes low, indicating variability in the data. However, the adjusted $R^2$, as a percentage of the maximum, generally showed a good fit for the respective equations (Table 3). In contrast to results for *S. zeamais* and *O. surinamensis*, mortality at 4 ppm rarely exceeded 40% (Fig. 3A–F). Mortality after 3 and 6 d of the exposure increased as concentration increased, but more gradually than was

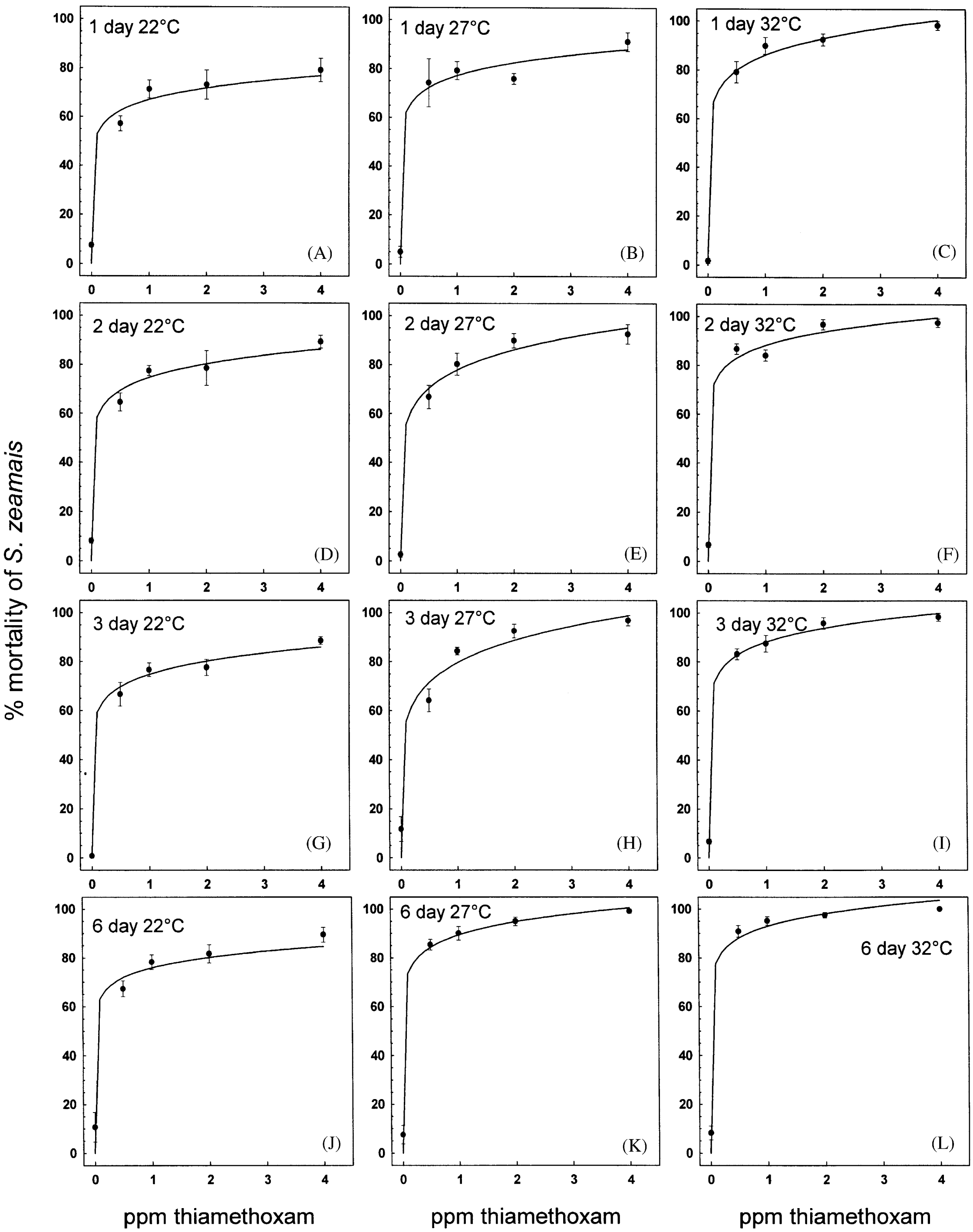


Fig. 1. (A–L) Mortality (mean ± SEM) of *Sitophilus zeamais* exposed for 1, 2, 3, or 6 d on maize treated with 0, 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam at three temperatures. Curve-fit lines are from equations in Table 1.

Table 2
Parameters (mean ± SE) for non-linear equations for mortality ($Y$) of *Oryzaephilus surinamensis* exposed for 1, 2, 3, or 6 d at 22°C, 27°C, or 32°C (temperature), on maize treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam ($x$)

| Exposure (d) | Temperature (°C) | $a$ | $b$ | $R^2$ | Max $R^2$ | % of max |
|---|---|---|---|---|---|---|
| 1 | 22 | 28.3 ± 3.4 | 0.502 ± 0.110[a] | 0.66 | 0.67 | 98.5 |
| | 27 | 32.3 ± 3.6 | 0.436 ± 0.104[a] | 0.66 | 0.67 | 98.5 |
| | 32 | 31.7 ± 4.2 | 0.549 ± 0.121[a] | 0.66 | 0.67 | 98.5 |
| 2 | 22 | 73.7 ± 4.1 | 70.7 ± 7.5[b] | 0.76 | 0.77 | 98.7 |
| | 27 | 79.2 ± 5.8 | 78.6 ± 10.5[b] | 0.67 | 0.69 | 97.1 |
| | 32 | 92.2 ± 4.2 | 88.5 ± 7.7[b] | 0.82 | 0.83 | 98.7 |
| 3 | 22 | 41.3 ± 4.8 | 0.518 ± 0.107[a] | 0.69 | 0.71 | 97.1 |
| | 27 | 98.7 ± 4.4 | 92.8 ± 8.1[b] | 0.83 | 0.85 | 97.6 |
| | 32 | 73.3 ± 3.0 | 0.229 ± 0.042[a] | 0.88 | 0.98 | 97.8 |
| 6 | 22 | 87.0 ± 4.3 | 94.8 ± 7.8[b] | 0.81 | 0.85 | 95.2 |
| | 27 | 111.4 ± 4.6 | 100.5 ± 8.3[b] | 0.84 | 0.88 | 95.4 |
| | 32 | 91.8 ± 2.1 | 0.094 ± 0.017[a] | 0.93 | 0.95 | 97.9 |

*Note*: Also included are the $R^2$ values of the equations, the maximum (max) $R^2$ that could be obtained from the data set, and the estimated $R^2$ as a percentage of the maximum.
[a] Non-linear equations were of the form $Y = ax^b$.
[b] Non-linear equations were of the form $Y = a - b\mathrm{e}^{-x}$.

observed for the other two species (Fig. 3G–L). Mortality also increased with temperature when *T. castaneum* were exposed for 3 and 6 d (Fig. 3G–L).

Few $F_1$ adults of any species were found in the treated maize; therefore, the $F_1$ values of each species in treated and untreated maize were analyzed separately. The number of $F_1$ adult *S. zeamais* in untreated maize varied significantly with temperature ($F = 14.2$; df = 2, 60), days of exposure ($F = 10.8$; df = 3, 60), and the temperature × exposure interaction ($F = 5.0$; df = 6, 60) ($P < 0.01$). At the 3- and 6-d exposures, more adults were produced at 27°C than at 22°C or 32°C (Table 4). Main effects were not significant ($P \geqslant 0.05$) in treated maize, and the number of $F_1$ adults averaged 0.2 ± 0.02. Main effects were not significant ($P \geqslant 0.05$) for adult *O. surinamensis* in untreated or in treated maize, and the overall averages were 10.5 ± 3.1 and 0.6 ± 0.07, respectively. Data for *T. castaneum* were not analyzed because this species did not reproduce on whole-kernel maize; the overall averages of $F_1$ adults in untreated and treated maize were only 0.6 ± 0.1 and 0.1 ± 0.02, respectively.

### 3.2. Experiment 2: evaluation as a protectant of hard red winter wheat seed

Main effects temperature ($F = 153.8$; df = 2, 240), exposure interval ($F = 622.3$; df = 3, 240), and application rate ($F = 532.3$; df = 4, 240), plus all interactions: temperature × exposure ($F = 6.8$; df = 6, 240), exposure × rate ($F = 33.2$; df = 12, 240), temperature × rate ($F = 11.6$; df = 8, 240), and temperature × exposure × rate ($F = 2.4$; df = 24, 240), were significant for mortality of *R. dominica* exposed on wheat treated with thiamethoxam ($P < 0.01$). Non-linear

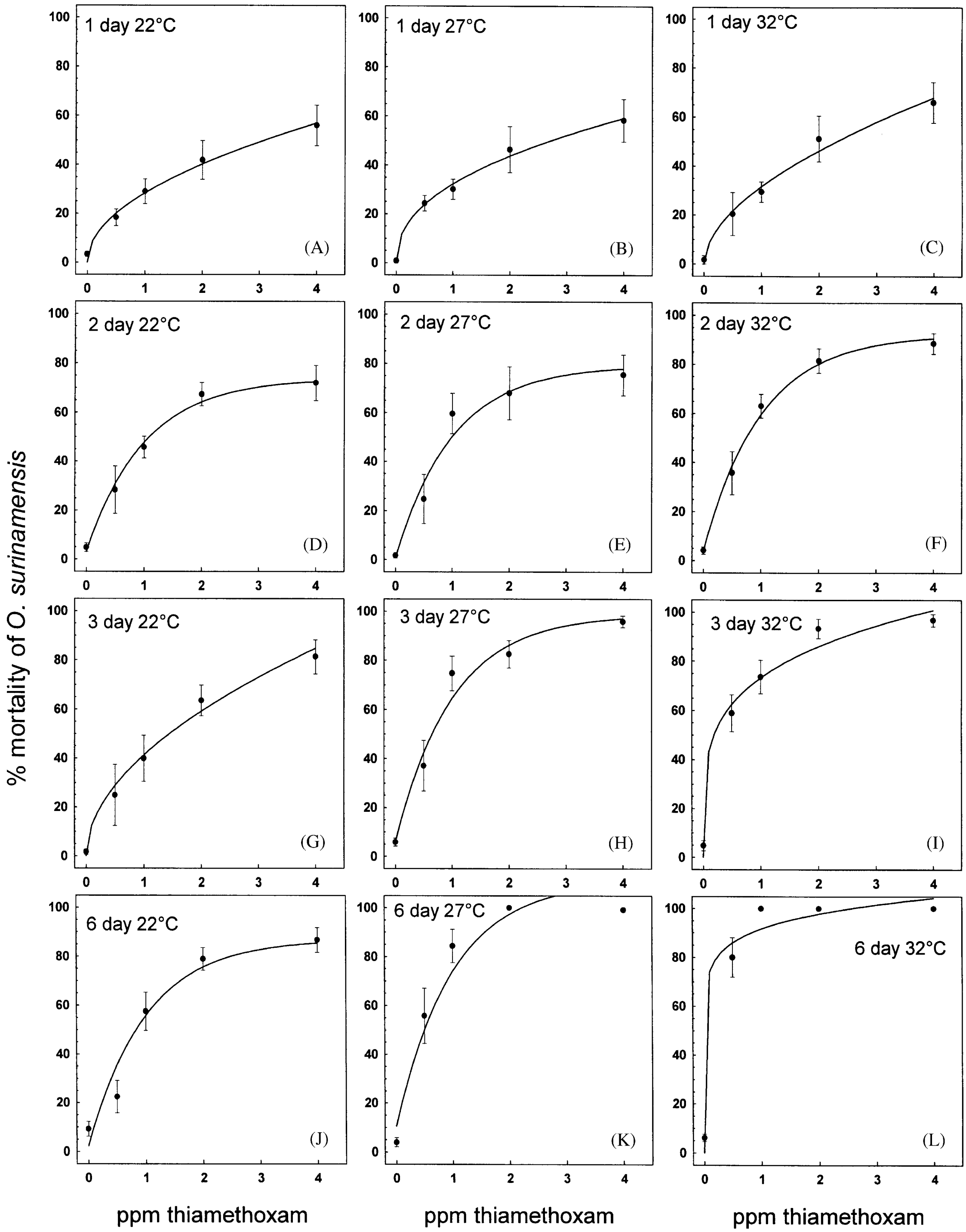


Fig. 2. (A–L) Mortality (mean ± SEM) of *Oryzaephilus surinamensis* exposed for 1, 2, 3, or 6 d on maize treated with 0, 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam at three temperatures. Curve-fit lines are from equations in Table 2.

Table 3
Parameters (mean ± SE) for linear equations[a] and non-linear equations[b] for mortality ($Y$) of *Tribolium castaneum* exposed for 1, 2, 3, or 6 d at 22°C, 27°C, or 32°C (temperature), on maize treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam ($x$)

| Exposure (d) | Temperature (°C) | $a$ | $b$ | $R^2$ | Max $R^{2c}$ | % of max |
|---|---|---|---|---|---|---|
| 1 | 22 | −0.6 ± 2.8 | 9.7 ± 1.4[a] | 0.64 | 0.65 | 98.5 |
| | 27 | 13.1 ± 2.0 | 0.434 ± 0.144[b] | 0.51 | 0.56 | 91.1 |
| | 32 | 8.8 ± 1.8 | 0.549 ± 0.190[b] | 0.44 | 0.50 | 88.0 |
| 2 | 22 | −0.8 ± 3.9 | 10.8 ± 1.9[a] | 0.54 | 0.56 | 96.4 |
| | 27 | 4.2 ± 3.0 | 9.6 ± 1.5[a] | 0.61 | 0.63 | 96.8 |
| | 32 | 26.3 ± 2.2 | 0.460 ± 0.080[b] | 0.77 | 0.79 | 97.5 |
| 3 | 22 | 7.4 ± 3.6 | 11.3 ± 1.8[a] | 0.59 | 0.64 | 92.2 |
| | 27 | 42.6 ± 2.8 | 0.027 ± 0.071[b] | 0.69 | 0.75 | 92.0 |
| | 32 | 61.2 ± 4.0 | 0.231 ± 0.069[b] | 0.74 | 0.75 | 98.7 |
| 6[c] | 22 | 69.4 ± 2.2 | 0.129 ± 0.033[b] | 0.90 | 0.90 | 100 |
| | 27 | 93.2 ± 1.6 | 0.081 ± 0.010[b] | 0.96 | 0.97 | 99.0 |

*Note*: Also included are the $R^2$ values of the equations, the maximum (max) $R^2$ that could be obtained from the data set, and the estimated $R^2$ as a percentage of the maximum.
[a] Linear equations $Y = a - bx$.
[b] Non-linear equations $Y = ax^b$.
[c] Regressions non-significant ($P \geqslant 0.05$) for 6-d exposure at 32°C.

equations were fitted to the data, and the adjusted $R^2$ values showed that the equations fitted the data extremely well (Table 5). Mortality of *R. dominica* exposed for 1 and 2 d did not exceed 50% (Fig. 4A–E), except for those exposed at 32°C (Fig. 4F). At each temperature, mortality generally increased with the 3- and 6-d exposures, and within each exposure interval, there was a gradual increase in mortality with increasing temperature (Fig. 4G–L). Mortality of *R. dominica* exposed for the maximum time of 6 d approached 100% at 27°C and 32°C.

Main effects temperature ($F = 62.8$; df = 2, 240), exposure interval ($F = 397.2$; df = 3, 240), and application rate ($F = 443.8$; df = 4, 240), plus the exposure × rate ($F = 27.2$; df = 12, 240), and temperature × rate ($F = 4.2$; df = 8, 240) interactions were significant for mortality of *S. oryzae* exposed on wheat treated with thiamethoxam ($P < 0.01$). The temperature × exposure ($F = 2.1$; df = 6, 240; $P = 0.06$) and the overall temperature × exposure × rate ($F = 1.0$; df = 24, 240; $P = 0.42$) interactions were not significant. Non-linear equations were fitted to the data as described for *R. dominica* (Table 6). Mortality of *S. oryzae* exposed on the treated wheat followed similar patterns to the results for *R. dominica*, and did not exceed 60% when beetles were exposed for 1 and 2 d (Fig. 5A–F). However, as exposure intervals increased to 3 d (Fig. 5G–I) and then to 6 d (Fig. 5J–L), mortality also increased, and approached 100% when beetles were exposed for 6 d at 27°C and 32°C.

Main effects temperature ($F = 179.8$; df = 2, 240), exposure interval ($F = 1770.7$; df = 3, 240), and application rate ($F = 296.8$; df = 4, 240), plus all interactions: temperature × exposure ($F = 66.7$; df = 6, 240), exposure × rate ($F = 117.2$; df = 12, 240), temperature × rate ($F = 10.9$;

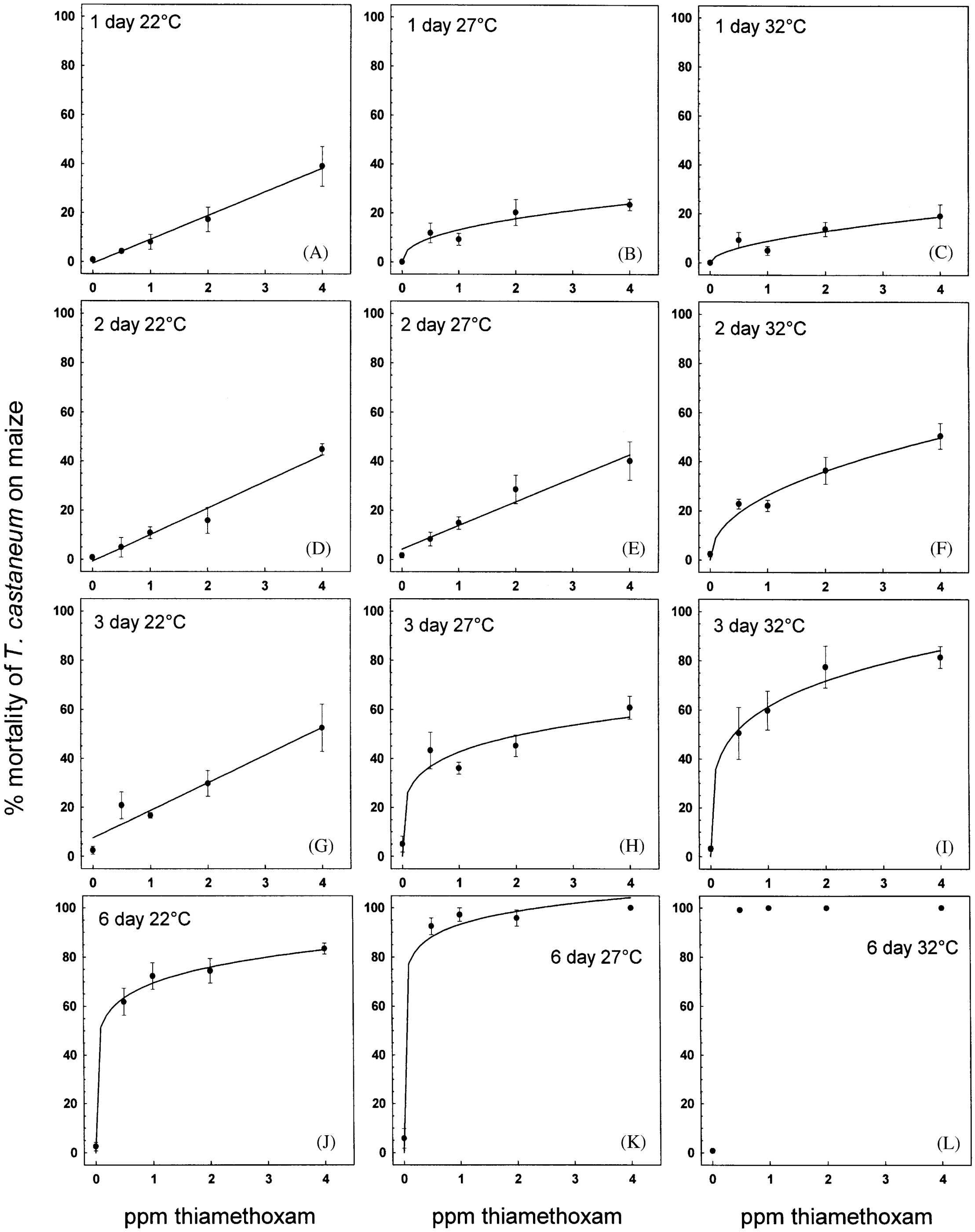


Fig. 3. (A–L) Mortality (mean ± SEM) of *Tribolium castaneum* exposed for 1, 2, 3, or 6 d on maize treated with 0, 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam at three temperatures. Curve-fit lines are from equations in Table 3.

Table 4
Mean number (±SEM) of adult $F_1$ *Sitophilus zeamais* in untreated maize[a]

| Temperature (°C) | Exposure (d) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 6 |
| 22 | 0.7±0.3a | 1.5±0.3a | 0.8±0.4b | 5.0±1.0b |
| 27 | 2.5±0.6a | 3.0±0.6a | 8.5±3.0a | 18.8±4.5a |
| 32 | 1.2±0.3a | 4.8±1.6a | 2.8±0.9b | 3.7±1.3b |

*Note*: Twenty mixed-sex adults were exposed for 1, 2, 3, or 6 d in vials containing 25 g maize, then the adults were removed and the vials were held for 8 weeks at 22°C, 27°C, and 32°C (temperature), 57% r.h.

[a] Means within columns followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan $k$-ratio $t$-test).

Table 5
Parameters (mean±SE) for non-linear equations for mortality ($Y$) of *Rhyzopertha dominica* exposed for 1, 2, 3, or 6 d at 22°C, 27°C, or 32°C (temperature), on wheat treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam ($x$)

| Exposure (d) | Temperature (°C) | $a$ | $b$ | $R^2$ | Max $R^2$ | % of max |
|---|---|---|---|---|---|---|
| 1 | 22 | 1.2±1.2 | 0.47±0.05[a] | 0.79 | 0.81 | 97.5 |
| | 27 | 36.9±2.4 | 37.3±4.5[b] | 0.75 | 0.82 | 91.6 |
| | 32 | 22.7±1.5 | 0.432±0.061[c] | 0.87 | 0.89 | 97.7 |
| 2 | 22 | 50.9±2.3 | 56.2±4.2[b] | 0.89 | 0.94 | 94.7 |
| | 27 | 55.4±3.1 | 79.3±5.6[b] | 0.77 | 0.82 | 93.9 |
| | 32 | 56.5±1.9 | 0.263±0.035[c] | 0.94 | 0.94 | 100 |
| 3 | 22 | 38.9±3.7 | 0.428±0.090[c] | 0.72 | 0.75 | 96.0 |
| | 27 | 71.3±2.4 | 0.078±0.019[c] | 0.87 | 0.88 | 98.8 |
| | 32 | 78.9±2.1 | 0.080±0.015[c] | 0.91 | 0.92 | 98.9 |
| 6 | 22 | 74.3±2.9 | 0.072±0.042[c] | 0.89 | 0.92 | 96.7 |
| | 27 | 90.7±1.5 | 0.090±0.012[c] | 0.97 | 0.97 | 100 |
| | 32 | 94.8±1.3 | 0.076±0.007[c] | 0.97 | 0.98 | 98.9 |

*Note*: Also included are the $R^2$ values of the equations, the maximum (max) $R^2$ that could be obtained from the data set, and the estimated $R^2$ as a percentage of the maximum.

[a] Non-linear equation $Y = a + be^x$.

[b] Non-linear equation $Y = a - be^{-x}$.

[c] Non-linear equation $Y = ax^b$.

df = 8, 240), and temperature × exposure × rate ($F = 6.2$; df = 24, 240) were significant for mortality of *T. castaneum* exposed on wheat treated with thiamethoxam ($P < 0.01$). However, these mortality data were extremely variable, similar to results for *T. castaneum* exposed on maize, with low maximum $R^2$ values in some temperature–exposure combinations (Table 7). Linear and non-linear equations were fitted to the data. Mortality of *T. castaneum* on wheat treated with thiamethoxam was far lower than corresponding mortalities of *R. dominica* and *S. oryzae*, and did not exceed 20% at the 1- and 2-d exposures (Fig. 6A–F). At 3 d exposure, mortality of *T.*

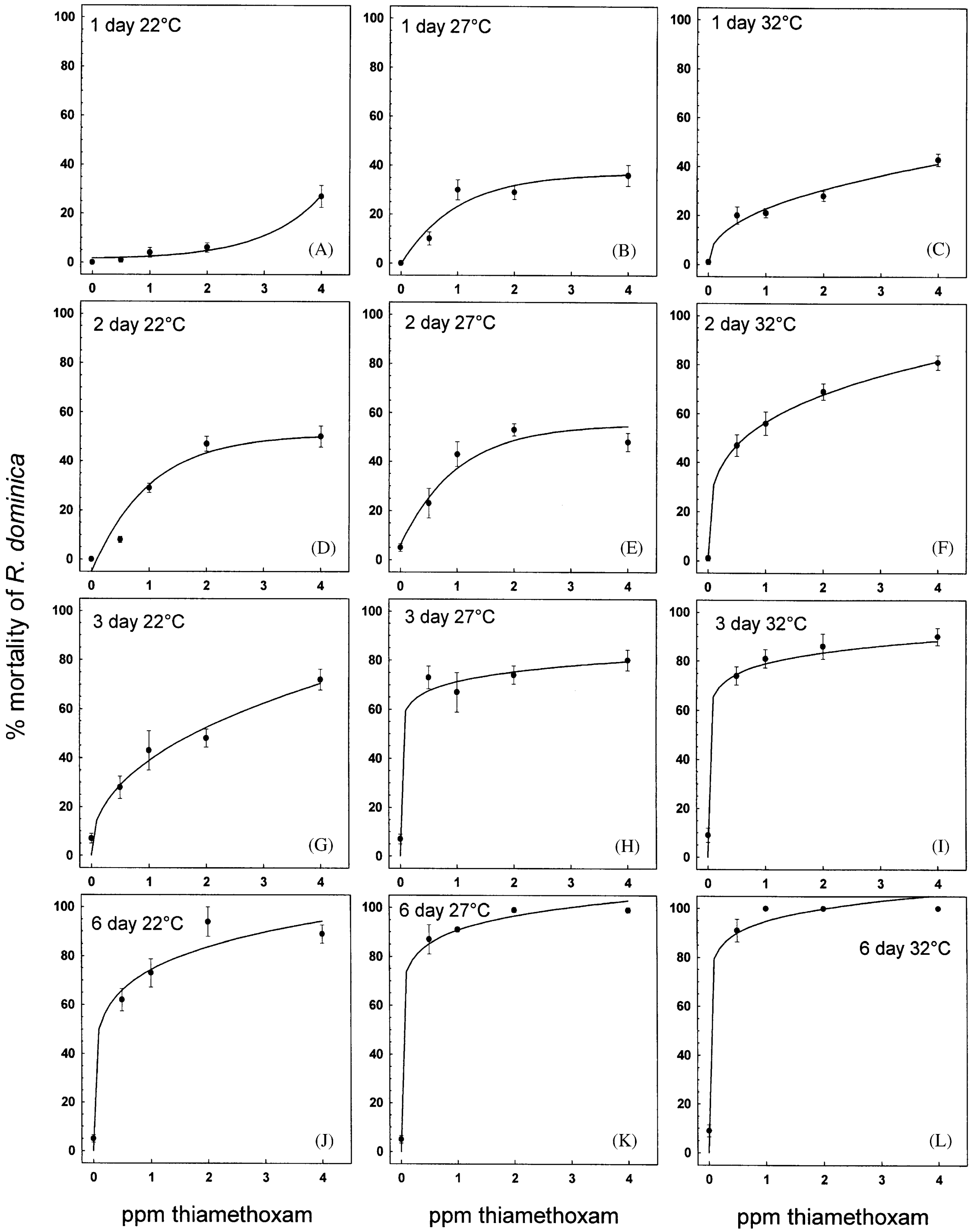


Fig. 4. (A–L) Mortality (mean ± SEM) of *Rhyzopertha dominica* exposed for 1, 2, 3, or 6 d on wheat treated with 0, 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam at three temperatures. Curve-fit lines are from equations in Table 5.

Table 6
Parameters (mean ± SE) for non-linear equations for mortality ($Y$) of *Sitophilus oryzae* exposed for 1, 2, 3, or 6 d at 22°C, 27°C, or 32°C (temperature), on wheat treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam ($x$)

| Exposure (d) | Temperature (°C) | $a$ | $b$ | $R^2$ | Max $R^2$ | % of max |
|---|---|---|---|---|---|---|
| 1 | 22 | 18.1 ± 1.5 | 0.394 ± 0.082[a] | 0.78 | 0.80 | 97.5 |
| | 27 | 23.7 ± 2.7 | 0.317 ± 0.113[a] | 0.62 | 0.63 | 98.4 |
| | 32 | 30.9 ± 2.8 | 0.241 ± 0.092 | 0.67 | 0.68 | 98.5 |
| 2 | 22 | 25.6 ± 2.5 | 0.454 ± 0.092[a] | 0.76 | 0.78 | 97.4 |
| | 27 | 36.7 ± 2.7 | 0.360 ± 0.071[a] | 0.82 | 0.83 | 98.7 |
| | 32 | 70.6 ± 3.1 | 63.4 ± 5.5[b] | 0.85 | 0.86 | 98.8 |
| 3 | 22 | 45.7 ± 2.4 | 0.336 ± 0.051[a] | 0.89 | 0.90 | 98.9 |
| | 27 | 82.9 ± 3.6 | 81.7 ± 6.5[b] | 0.87 | 0.89 | 97.7 |
| | 32 | 68.8 ± 2.4 | 0.173 ± 0.038[a] | 0.91 | 0.94 | 96.8 |
| 6 | 22 | 75.3 ± 2.4 | 0.117 ± 0.031[a] | 0.91 | 0.91 | 100 |
| | 27 | 91.7 ± 2.1 | 0.091 ± 0.062[a] | 0.94 | 0.96 | 97.9 |
| | 32 | 94.5 ± 1.6 | 0.090 ± 0.012[a] | 0.97 | 0.99 | 97.9 |

*Note*: Also included are the $R^2$ values of the equations, the maximum (max) $R^2$ that could be obtained from the data set, and the estimated $R^2$ as a percentage of the maximum.
[a] Non linear equations $Y = ax^b$.
[b] Non-linear equations $Y = a - be^{-x}$.

*castaneum* exposed at 22°C and 27°C did not exceed 40% (Fig. 6G–I), and mortality approached 100% only when beetles were exposed for 6 d at 32°C (Fig. 6L). As with the other exposures, there was a clear indication of increasing mortality with increasing temperature.

Few $F_1$ adults of any species were found in the treated wheat, similar to the results for treated maize, so the $F_1$ values of each species in treated and untreated wheat were analyzed separately. Main effects temperature ($F = 138.0$; df = 2, 48) and days of exposure ($F = 23.6$; df = 3, 48), plus the temperature × exposure interaction ($F = 7.9$; df = 6, 48) were significant ($P < 0.01$) for the number of adult $F_1$ *R. dominica* in untreated wheat. Few adults were produced at 22°C, and at the 3- and 6-d exposures, more adults were produced at 32°C than at 22°C or 27°C (Table 8). Main effects temperature ($F = 27.8$; df = 2, 48) and days of exposure ($F = 14.6$; df = 3, 48) were significant at $P < 0.01$ for the number of adult $F_1$ *S. oryzae* in untreated wheat. The temperature × exposure interaction was not significant ($F = 1.8$; df = 6, 48; $P = 0.11$). At the 1- and 2-d exposures, there were no differences in the number of $F_1$ adults at 27°C and 32°C, but at the 3- and 6-d exposures more adults were produced at 27°C than at 32°C (Table 8). Data for adult $F_1$ *T. castaneum* were not analyzed because few adults were produced on either untreated or treated wheat.

Main effects temperature ($F = 10.8$; df = 2, 192) and rate ($F = 3.0$; df = 3, 192; $P = 0.03$) were significant at $P < 0.01$ for the number of adult $F_1$ *R. dominica* in wheat treated with thiamethoxam. The main effect exposure was not significant ($F = 0.3$; df = 3, 192; $P = 0.24$), nor were any interactions significant: temperature × exposure ($F = 0.7$; df = 6, 192; $P = 0.65$), exposure × rate ($F = 0.6$; df = 9, 192; $P = 0.82$), temperature × rate ($F = 0.7$; df = 6, 192; $P = 0.64$), and the

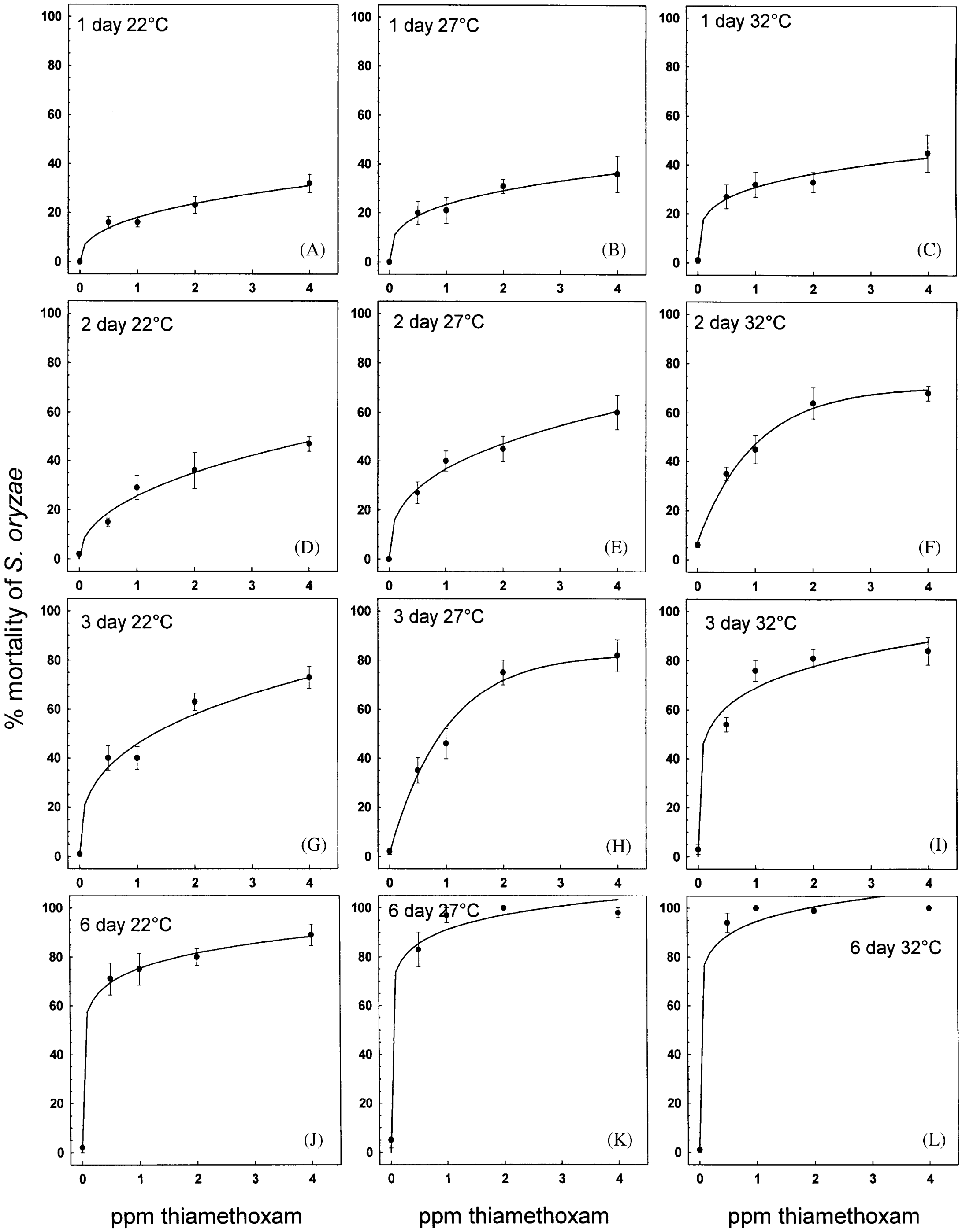


Fig. 5. (A–L) Mortality (mean ± SEM) of *Sitophilus oryzae* exposed for 1, 2, 3, or 6 d on wheat treated with 0, 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam at three temperatures. Curve-fit lines are from equations in Table 6.

Table 7
Parameters (mean ± SE) for linear and non-linear equations for mortality ($Y$) of *Tribolium castaneum* exposed for 1, 2, 3, or 6 d at 22°C, 27°C, or 32°C (temperature), on wheat treated with 0, 0.5, 1.0, 2.0, or 4.0 ppm thiamethoxam ($x$)[b]

| Exposure (d) | Temperature (°C) | $a$ | $b$ | $R^2$ | Max $R^2$ | % of max |
|---|---|---|---|---|---|---|
| 1[a] | 22 | −0.3 ± 0.8 | 1.8 ± 0.4[b] | 0.46 | 0.47 | 97.8 |
| | 27 | 2.8 ± 1.1 | 0.600 ± 0.362[c] | 0.24 | 0.26 | 92.3 |
| 2[a] | 22 | 1.8 ± 1.0 | 0.778 ± 0.482[c] | 0.23 | 0.25 | 92.0 |
| | 32 | 0.2 ± 1.4 | 2.9 ± 0.7[b] | 0.46 | 0.53 | 86.7 |
| 3 | 22 | 1.2 ± 2.0 | 4.8 ± 1.0[b] | 0.52 | 0.55 | 94.5 |
| | 27 | 12.2 ± 1.7 | 0.590 ± 0.122[c] | 0.69 | 0.73 | 94.5 |
| | 32 | 37.7 ± 1.7 | 0.340 ± 0.047[c] | 0.91 | 0.94 | 96.8 |
| 6 | 22 | 49.8 ± 2.5 | 0.201 ± 0.053[c] | 0.86 | 0.86 | 100 |
| | 27 | 77.8 ± 2.0 | 0.139 ± 0.027[c] | 0.95 | 0.95 | 100 |
| | 32 | 91.8 ± 1.6 | 0.340 ± 0.047[c] | 0.91 | 0.94 | 96.8 |

*Note*: Also included are the $R^2$ values of the equations, the maximum (max) $R^2$ that could be obtained from the data set, and the estimated $R^2$ as a percentage of the maximum.

[a] Regressions non-significant ($P \geqslant 0.05$) for 1-d exposure at 32°C and 2-d exposure at 27°C.

[b] linear equations $Y = a - bx$.

[c] Non-linear equations $Y = ax^b$.

temperature × exposure × rate ($F = 0.4$; df = 18, 192; $P = 0.98$). However, the numbers of $F_1$ adults in the treated wheat were so low that when rate was analyzed separately by both regression analysis and multiple range tests, regressions with insecticide concentration as the independent variable were not significant ($P \geqslant 0.05$). Furthermore, multiple comparisons were not significantly different with respect to concentration ($P \geqslant 0.05$) even though the overall ANOVA was significant. When data were combined for concentration and analyzed for differences among temperatures at each of the exposure intervals, there were no significant differences ($P \geqslant 0.05$). The combined average for the overall number of $F_1$ adults on the treated wheat was only 0.5 ± 0.7.

Main effects temperature ($F = 6.0$; df = 2, 192) and rate ($F = 20.8$; df = 3, 192; $P = 0.03$) were significant ($P < 0.01$) for the number of adult $F_1$ *S. oryzae* in wheat treated with thiamethoxam. Main effect exposure was not significant ($F = 0.1$; df = 3, 192; $P = 0.95$), nor were any interactions significant: temperature × exposure ($F = 0.8$; df = 6, 192; $P = 0.54$), exposure × rate ($F = 0.3$; df = 9, 192; $P = 0.96$), temperature × rate ($F = 1.4$; df = 6, 192; $P = 0.23$), or the temperature × exposure × rate ($F = 0.4$; df = 18, 192; $P = 0.97$). When data were combined for exposure intervals, there was no significant difference ($P \geqslant 0.05$) among temperatures. The average number of $F_1$ adult *S. oryzae* in wheat treated with 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam was 2.8 ± 0.05, 1.4 ± 0.2, 0.2 ± 0.07, and 0.2 ± 0.06, respectively.

## 4. Discussion

*Tribolium castaneum* appeared to be the least susceptible species evaluated for susceptibility to thiamethoxam. Although adult *R. dominica*, *S. oryzae*, and *S. zeamais* are primary pests of stored

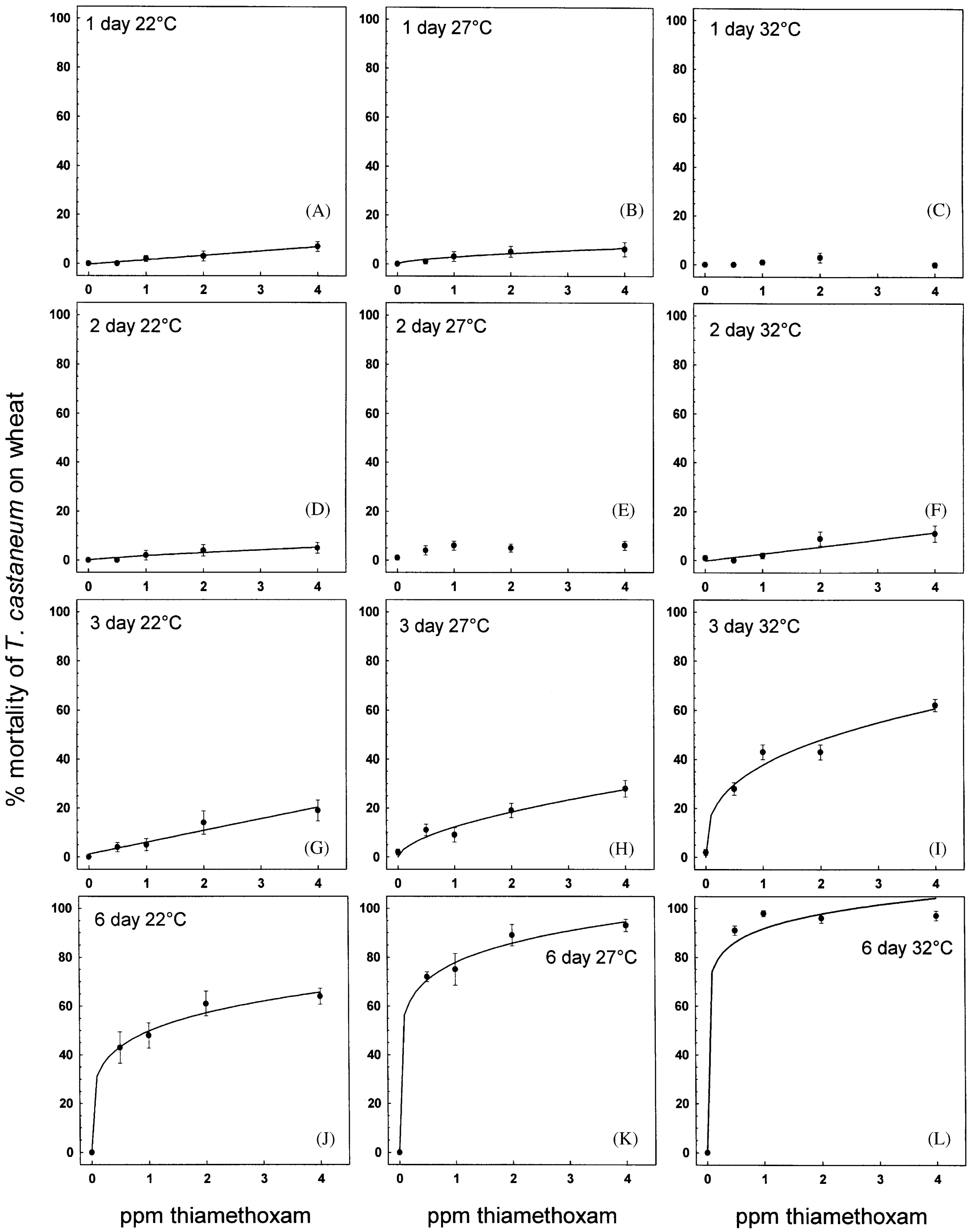


Fig. 6. (A–L) Mortality (mean ± SEM) of *Tribolium castaneum* exposed for 1, 2, 3, or 6 d on wheat treated with 0, 0.5, 1.0, 2.0, and 4.0 ppm thiamethoxam at three temperatures. Curve-fit lines are from equations in Table 7.

Table 8
Mean number (±SEM) of adult $F_1$ *Rhyzopertha dominica* and *Sitophilus oryzae* in untreated wheat[a]

| Temperature (°C) | Exposure (d) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 6 |
| *R. dominica* | | | | |
| 22 | 0.2±0.2b | 0.4±0.2b | 0.0±0.0c | 0.6±0.2c |
| 27 | 32.0±6.9a | 60.0±7.7a | 60.8±13.6b | 81.2±14.2b |
| 32 | 30.4±4.9a | 70.6±2.7a | 97.6±2.7a | 132.2±8.0a |
| *S. oryzae* | | | | |
| 22 | 6.2±3.7b | 6.0±2.9b | 8.8±3.7c | 25.8±11.2c |
| 27 | 22.2±7.3a | 39.0±6.4a | 51.8±6.1a | 83.0±7.8a |
| 32 | 19.0±6.9a | 36.8±7.1a | 32.8±9.4b | 51.6±8.3b |

*Note*: Twenty mixed-sex adults were exposed for 1, 2, 3, or 6 d in vials containing 25 g wheat, then the adults were removed and the vials were held for 8 weeks at 22°C, 27°C, and 32°C (temperature), 57% r.h.

[a] For each species, means within columns followed by the same letter are not significantly different ($P \geqslant 0.05$, Waller–Duncan $k$-ratio $t$-test).

grains, adult *T. castaneum* can be more difficult to kill than the primary pests or adults of other secondary pests of stored commodities. In tests with organophosphorus insecticides, mortality of *T. castaneum* is often less than mortality of *S. oryzae* when both are exposed at equivalent concentrations (LaHue, 1977a, b; Bengston et al., 1980) or the same post-treatment intervals (Thomas et al., 1987; White et al., 1997). Research with inert dusts such as diatomaceous earth has also shown that *T. castaneum* is harder to kill than *S. oryzae* (Korunic, 1998). However, both *S. oryzae* and *S. zeamais* are more tolerant than either *T. castaneum* or *R. dominica* when exposed to pyrethroids, and the order of susceptibility is apparently reversed (Bengston et al., 1987; Samson and Parker, 1989; Arthur, 1992, 1994a, b).

Results of these studies with maize and wheat also show a positive effect of exposure interval on mortality of insects exposed to thiamethoxam, which is expected (because the longer an insect is exposed on treated seed, the more likely it is to die). Mortality of *T. castaneum* exposed to thiamethoxam for 1 and 2 d was usually far less than mortality of *S. zeamais* on maize and *S. oryzae*, or *R. dominica* on wheat, and differential results among species often occur when insects are exposed for equivalent time intervals to specific concentrations. In a recent test with ethiprole, an insecticide that acts on the GABA receptor of insects, a 1-week exposure interval killed all *S. oryzae* exposed on wheat and *S. zeamais* exposed on maize treated with combination treatments of ethiprole plus other insecticides, but some *T. castaneum* were still alive after a 2-week exposure interval (Arthur, 2002). If insects are exposed for short time intervals on treated surfaces they could potentially survive the exposure if they could escape, which would not occur if they were continually exposed as in bulk seed. The degree of survival would depend on the insecticide concentration, characteristics of the insecticide, and the susceptibility of the individual insect species making the interaction between concentration and exposure interval important when assessing the value or efficacy of insecticides.

The effect of temperature on insecticide toxicity has been documented for various classes of insecticides. Organophosphate toxicity generally increases with temperature, while toxicity of some pyrethroids has been negatively correlated with increasing temperature (Hinks, 1985; Grafius, 1986; Thaung and Collins, 1986; Subramanyam and Cutkomp, 1987; Johnson, 1990; Arthur, 1999). Some tests show a positive response toxicity to diatomaceous earth with temperature, while others show a neutral or negative effect (Arthur, 2000). In these tests with thiamethoxam, insect mortality increased with temperature, which could be a function of increased insect movement leading to increased contact with the toxicant on treated grain and to increased respiration and uptake of thiamethoxam in response to the increased temperature.

One of the justifications for selecting the concentrations used in these studies was to use rates that would be equivalent to current insecticides used to control stored-product beetles, so as to compare the results of those chemicals and with others being evaluated for post-harvest use. Currently, the only insecticides labeled for direct application to bulk wheat stored in bins or silos are the organophosphates chlorpyrifos-methyl and malathion, which are labeled at 6 and 8 ppm, respectively. The product label for chlorpyrifos-methyl does not specify control of *R. dominica*, a major pest of stored grains, while malathion resistance in major stored-grain insects is a worldwide problem, including the United States (Subramanyam and Hagstrum, 1996). Thiamethoxam applied at 1–4 ppm gave 90–100% control when *R. dominica* and *S. oryzae* were exposed for 3 and 6 d on treated wheat held at 27°C and 32°C, and would seem to be an effective protectant of seed wheat or bulk wheat. Similarly, the organophosphate pirimiphos-methyl is labeled for 6–8 ppm on stored maize, but thiamethoxam controlled *S. zeamais*, an internal pest of stored maize, at rates of 1–4 ppm. Tests with the bacterial pesticide Spinosad gave complete control of *R. dominica* and *S. oryzae* exposed on different classes of wheat treated with 1 and 3 ppm, while higher rates were required to give equivalent control of *T. castaneum* (Fang et al., 2002).

Our studies show that thiamethoxam would be an effective protectant of seed maize and seed wheat, or of bulk commodities stored in farm bins and elevator silos. The actual level of control would be dependant on the interaction of physical and biological factors such as target insect species, application rate, time interval in which insects were exposed on the treated commodity, and the temperature at which the insects were exposed. Other factors that could affect toxicity include the specific grain to be treated, its moisture content, and the time treated grain is in storage before it is challenged by insect infestation. Potential application rates would be comparable to those insecticides that are currently labeled for control of stored-product beetles in seed and bulk commodities.

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