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Forced hot-air quarantine treatment to control *Acanthoscelides pallidipennis* in seeds of false indigo (*Amorpha fruticosa)*

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ABSTRACT

To establish a schedule for phytosanitary treatment of the North American bruchid *Acanthoscelides pallidipennis* (Motschulsky) in false-indigo (*Amorpha fruticosa* L.) seeds, the dose response and confirmatory tests were conducted in a forced hot-air chamber. In the dose-response tests, a minimum heating time required for 100% mortality of the bruchid was 80, 32, and 24 min at temperature and humidity combinations of 56°C-70% r.h. 58°C-70% r.h., and 60°C-70% r.h. respectively. The thermal death kinetic model was used to predict the minimum time for 99% and 99.9968% mortality at the 95% confidence level. In the confirmatory tests, no adult emerged from an estimated 132,000 fourth stage larvae in all the seeds (24 kg) treated at 60°C-70% r.h. for 40 min, resulting in the disinfestation efficacy of 99.9977% at the 95% confidence level. There was no impact on the germination of the treated seeds. Therefore, the minimum heating time of 40 min at 60°C-70% r.h. could be recommended as the phytosanitary schedule for the controlling of *A. pallidipennis* in false-indigo seeds.

Key words: *Acanthoscelides pallidipennis*, *Amorpha fruticosa,* Phytosanitary heat treatment, Thermal death kinetic

The North American bruchid, *Acanthoscelides pallidipennis* (Motschulsky), is a major pest infesting the false indigo (*Amorpha fruticosa* L.) seeds both in field and postharvest storage (Gao, 1991). The infestation rate is 20–40% in the field, as a result, an average of 65% of the infested seeds lose their germination capacity (Wan, 1989; Wan and Xu, 1991; Yang et al., 1992). At present, *A. pallidipennis* is listed as a quarantine pest in China, Japan and Korea, and the infested seeds must undergo phytosanitary treatments (Wan and Xu, 1991; Tude, 2001). However, a very high dosage is required for achieving 100% mortality by fumigating with methyl bromide and sulfuryl fluoride at the temperatures below 10°C (Zhan et al., 2011), and the bruchid was not 100% killed by phosphine fumigating with a dosage of 4 g/m^3 for 15 d at 19.4°C (Lin et al., 1988). Therefore, an alternative quarantine treatment is needed to be developed. High temperature forced air treatment (HTFA) has been found to be effective for controlling a number of post-harvest pests in commodities (Sharp, 1993; Hallman, 1994; Canovai et al., 2001; Johnson et al., 2004). Here, we report the efficacy of HTFA against the bruchid in false indigo seeds.

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MATERIALS AND METHODS

Infested seed and testing insects

The naturally infested seeds of *A. fruticosa*, which were collected from Wendeng County, Shandong, China, were kept in a storeroom (5–13°C) for slowly drying to the moisture content of 9.5–10.0%. Then the infested seeds were used for the dose–response and confirmatory tests.

HTFA chamber

A HTFA chamber (Chongqing Well Experiment Equipment Co. Ltd, ChongQing, China) was used for conducting all the tests. The dimension of inner holding room was 510 mm × 760 mm × 990 mm and it could be separated freely by stainless steel mesh plates.

The temperature and relative humidity (–5°C–60°C, accuracy: 0.1°C; 30–98% r.h., accuracy: ±3%) in the holding room could be controlled precisely. The circulating air rate was 0–2 ms^{-1} and was adjusted to 1 ms^{-1} during the treatment. Testo type K thermocouples (-10°C–1,000°C, accuracy: ±0.3°C), which were calibrated before each experiment, were connected to a Testo 177-T4 data logger (Lenzkirch, Germany) to monitor and record the temperatures.

Dose-response tests

The dose-response tests were designed to treat the infested seeds at constant relative humidity of 70% r.h., the heating time was 30, 40, 50, 60, 70, 80, 90 and 100 min at 56°C; 8, 12, 16, 20, 24, 28, 32 and 36 min at 58°C; and 4, 8, 12, 16, 20, 24, 28 and 32 min at 60°C. In each treatment, 100 g false indigo seeds were distributed. In a single layer at the bottom of a mesh basket and heated to the target time. Air temperature around the seeds was recorded. After treatment, the seeds were air cooled, weighed, and stored for 7 days at room temperature. Then, the mortality of the bruchids was checked by counting at least 100 larvae for each treatment.

Confirmation tests

To validate the Probit 9 lethal time ($LT_{99.9968}$) estimated by a kinetic model, at least 93,636 insects should be tested with no survivor in the confirmatory tests (Couey and Chew, 1986). A total of 24 kg infested seeds were treated at 60°C–70% r.h. for 40 min in the 4 confirmatory tests. In each treatment, 6 kg seeds were put onto a basket with mesh used to form a 6 cm thickness layer, then the thermocouples were inserting into the seed layer and fixed at the depth of 0, 20, 40, and 60 mm. After treatment, the seeds at the depth of 0–20 mm, 20–40 mm and 40–60 mm were stored separately in a storeroom for almost 6 months to investigate the mortality (no adult emergence) of the bruchid.

Germination test

For conducting germination tests, plump seeds were heated to the longest heating time in the dose-response tests, were used for the germination tests. Each germination treatment had 4 replicates with 100 g seeds. After treatment, the seeds were immersed in boiled water, stirred, soaked overnight and incubated at 23–26°C for germination. The germination was determined within 2 weeks (Xu, 2011).

Data analysis

The dose-response mortality data, adjusted by Abbott's formula (Abbott, 1925), were linearized by thermal kinetic transformation. The thermal kinetic model had the form:

$$(\mathrm{Log}N_0 - \mathrm{Log}N)^a = k*t + c \quad \ldots(1)$$

where N_0 is the initial number of larvae treated, N is the adjusted number of surviving larvae, a is a thermal death kinetic order; t is heating time in min, c is the intercept (Alderton and Snell, 1970; Jang, 1986). Upper and lower 95% CL were calculated for each lethal time with the methods of Zhang (1988). Each regression was tested for heterogeneity by using Pearson chi-square statistic (Zhang, 1988). Data used in the analyses included any heating time causing mortality between 0 and 100%, and the shortest time causing 100% mortality.

For the confirmatory tests, the mortality proportion ($1 - P_u$) associated with treating a number of insects with zero survivors is given by the equation:

$$1 - P_u = (1 - C)^{1/n} \quad \ldots(2)$$

where C in the confidence level, P_u is the acceptable level of survivorship (as a proportion) and n is the number of testing insects (Couey and Chew, 1986).

RESULTS AND DISSCUSION

Stages of testing insects

The larval infestation rate checked by dissecting the false-indigo seed was about 5.6%, most of the larvae were in the final larval stage, the fourth instar. A number of investigations have revealed that the final larval stage may be the most tolerant stage for beetles by heat treatment (Canovai et al., 2001; Johnson et al., 2004; MeKasha, 2006). Therefore, the infested seeds could be used for conducting treatments directly, and there were 800 g seeds and more than 4,000 larvae used for each temperature and humidity combination.

Thermal death of Acanthoscelides pallidipgennis *larvae*

The thermal death kinetic order was compared and the results showed that the 0.5 order model produced the largest coefficients of determination (R^2) values for all the three treatments, followed by 1, 0, 1.5, and 2 orders. Thus, the 0.5 order model was used for further calculations in this investigation.

Results from the analysis of dose-response mortality data showed that the thermal kinetic model was well fitted to the thermal mortality data for each temperature and humidity combination as the coefficients of determination were larger than 0.9 (Table 1; Fig.1). Temperatures increases from 56 to 60°C without constant relative humidity led to dramatic decrease

Table 1 Lethal times (min) obtained by the 0.5 kinetic model to achieve 99% and 99.9968% mortality for the fourth instar larvae of *Acanthoscelides pallidipgennis* and germination rate at three temperature and humidity combinations*

Temperature and humidity combination	Thermal death constants		Lethal time (min) (95%CL)		R^2	Germination (Mean±SD%)
	K (±SE)	χ^2	$LT_{99.9968}$	LT_{99}		
56°C –70% r.h.	0.01556 (0.0013)	3.069	122.3 (106.5–138.2)	76.9 (70.6–83.3)	0.9679	87.8±5.9a
58°C –70% r.h.	0.04744 (0.0021)	2.965	46.4 (43.2–49.5)	31.5 (29.9–33.1)	0.9903	85.8±6.7a
60°C –70% r.h.	0.0586 (0.0031)	1.814	33.8 (30.8–36.9)	21.8 (20.3–23.3)	0.9891	93.7±2.3a
Control						92.8±2.1a

*R^2 is the coefficient of determination; *germination data were subjected to one-way analysis of variance (ANOVA), means were compared by Tukey's multiple comparison tests at the 5% confidence level

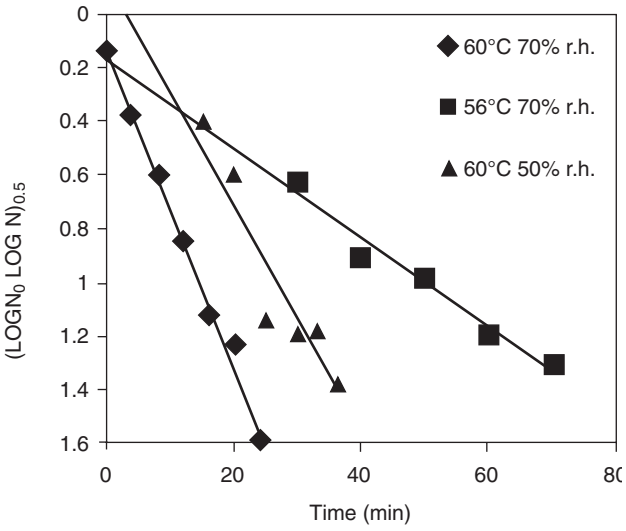

Fig. 1. Thermal death curve for the fourth instar larvae of *Acanthoscelides pallidipgennis* at three temperature and relative humidity combinations by the 0.5 kinetic model

of $LT_{99.9968}$ from 113.38 min to 32.49 min, indicating that the bruchid larvae is very sensitive to temperature changes (Fig. 1). Therefore, much shorter exposure time was needed in the combination with higher temperature (Fig. 1). The germination test (Table 1) also revealed that there was no significant difference in germination even when seeds were treated with the longest exposure time in each temperature and humidity combination ($F=2.646$, $P=0.0967$). Then, the highest temperature of 60°C was used for the following confirmatory tests.

Confirmatory tests

To validate the estimated $LT_{99.9968}$ of 33.8 (30.8–36.9) min (Table 1), a heating time of 40 min was used as the target heating time during confirmatory tests, as 3–5 min was needed for the chamber to reach the required temperature and relative humidity. The result showed that the heating times for the temperature to reach the desired level (60°C) decreased from the upper layer to bottom layer. The longest time of 35.5 to 37 min was required for the upper layer, followed by the middle and bottom layer in all the 4 replication tests (Table 2). The shortest exposure time of 30.5 min appeared in bottom layer (Table 2), but it is much closer to the lower bound (30.8 min) of $LT_{99.9968}$ at the 95% CL (Table 1).

No adult emerged from an estimated 132,000 larvae in all the treated seeds, on the contrary, a total of 26,492, 22,576, and 26,280 adults were counted from the three layers in control (6 kg) respectively. The great number of adults present in the control indicated that the larvae developed one more generation during the 6 months storage (Sadakiyo and Ishihara, 2011). Thus, the estimation by thermal death kinetic model was validated in the confirmatory tests. The disinfestation efficacy calculated by Equation (2) is 99.9977% at the 95% confidence level (Couey and Chew, 1986). The maximum heating duration used in the confirmatory tests may be the minimum duration required for the approved treatment. Therefore, an exposure time of 40 min at 60°C-70% r.h., providing the disinfestation efficacy of 99.9977%, could be suggested as the minimum exposure time for phytosanitary heat treatment of fourth-instar *A. pallidipennis* in false-indigo seeds.

To establish a phytosanitary treatment schedule, the commodity should be tolerant to the treatment. There were no significant reductions in germination when the false-indigo seeds were treated in confirmatory tests or longer exposure times occurred in dose-response tests, comparing with the untreated seeds (Tables 1, 2). As the fourth instar is the most developed stage in seed and it may develop and reproduce at suitable temperature during storage (Sadakiyo and Ishihara, 2011), the treatment schedule proposed in this study could be used for pre-storage treatment to protect seeds from the infestation, and providing quarantine security of shipped seeds.

Table 2 Number of *Acanthoscelides pallidipgennis* adults emerged from treated seed, exposure duration above 60°C, and germination rate in the confirmatory tests*

		Treatment replications				Control
		rep.1	rep.2	rep.3	rep.4	
No. larvae estimated		33,000	33,000	33,000	33,000	33,000
Exposure duration exceed 60°C (min)	UL	35.5	36	36	37	0
	ML	34	34	33.5	35	0
	BL	32	34	30.5	34.5	0
No. adult emerged	UL	0	0	0	0	26,492
	ML	0	0	0	0	22,576
	BL	0	0	0	0	26,280
Germination* (Mean ± SD%)	UL	94.2 ± 2.5a				92.4 ± 1.8a
	ML	93.5 ± 3.4a				90.8 ± 2.4a
	BL	93.2± 4.2a				93.8 ± 3.7a

UL, ML, and BL means the upper, middle, and bottom layer respectively

*Germination data were subjected to one-way analysis of variance (ANOVA), means were compared by Tukey's multiple comparison tests at the 5% confidence level

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