
Disinfestation of Hessian fly puparia in small rectangular hay bales using a laboratory heat treatment unit

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Opoku, A., Sokhansanj, S., Crerar, W.J., Tabil, L.G. and Whistlecraft, J.W. 2002. **Disinfestation of Hessian fly puparia in small rectangular hay bales using a laboratory heat treatment unit.** Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **44**: 3.27-3.33. Tests were conducted to verify the disinfestation of small rectangular hay bales (mixture of alfalfa/bromegrass) in a laboratory heat treatment unit. Cavities were made on top of the hay bales and Hessian fly- (*Mayetiola destructor* (Say)) infested wheat seedlings contained in polyester mesh bags were placed in them. For each bale tested, three mesh bags of Hessian fly puparia were used. A bale inserted with the mesh bags was thermally treated. The experimental tests were done in a completely random order. Ambient air (20 – 27°C) was circulated through three control mesh bags containing infested wheat seedlings. An estimated number of 1220 puparia were used for each disinfestation test and the control. The density of the bales ranged from 113 to 148 kg/m³, and the initial moisture content ranged from 8.5 to 9.9% wet basis. The average temperatures used in the heat treatment ranged from 73 to 76°C, and the average relative humidity ranged from 39 to 53%. The thermally treated insect bags and the controls were sent for emergence test. About 241 insects survived out of the approximate number of 1220 puparia used as control. This represented a pre-treatment viability of about 20%, which was unexpectedly low. The thermal treatment ensured a total mortality of the Hessian fly puparia. This study has shown that thermal treatment could be used to disinfest hay bales to meet quarantine and phytosanitary regulations. **Keywords:** Hessian fly, disinfestation, baled hay, forage, thermal treatment, quarantine, heat treatment.

Des tests furent faits en laboratoire afin de déterminer l'efficacité de la désinfestation de petites balles de foin (mélange luzerne/brome) dans des appareils de traitement thermique. Des semis de blé infestés de mouches de Hesse, contenus dans de petits sacs de polyester à maille, furent placés dans des trous pratiqués au sommet des balles de foin. Trois sacs de pupes de mouche de Hesse furent insérés dans chacune des balles testées. Les balles infestées reçurent un traitement thermique. Les tests furent réalisés dans un ordre aléatoire. Trois sacs infestés de mouches dans lesquels on fit circuler de l'air à température ambiante (20 – 27°C) servirent de contrôle. Dans chacun des tests de désinfestation et dans le contrôle, 1220 pupes furent utilisées. La densité des balles de foin allait de 113 à 148 kg/m³, et la teneur en eau initiale s'étendait de 8.5 à 9.9% (base humide). Les températures moyennes lors du traitement thermique allaient de 73 à 76°C, et l'humidité relative moyenne de 39 à 53%. Des tests d'émergence furent faits sur les sacs infestés traités thermiquement et sur les contrôles. Environ 241 insectes survécurent sur les 1220 qui avaient été placés dans le contrôle. Le taux de survie après le pré-traitement était donc d'environ 20%, un taux plus faible que ce qui avait été

envisagé. Le traitement thermique a permis une élimination complète des pupes de mouche de Hesse. Cette étude a permis de démontrer qu'un traitement thermique peut désinfester les balles de foin et ainsi respecter les mesures de quarantaine et la réglementation phytosanitaire. **Mots clé:** mouche de Hesse, désinfestation, balle de foin, fourrage, traitement thermique, quarantaine.

INTRODUCTION

Phytosanitary and quarantine regulations stipulate that shipments of compressed baled hay be inspected for or be disinfested of Hessian fly, *Mayetiola destructor* (Say), contamination. Plants such as wheat, barley, and *Agropyron* spp. grasses are host to the Hessian fly. The infested plants growing in hay fields as weeds may be introduced as contaminants when the hay is cut and baled. Visual inspections are usually conducted by quarantine officers to identify host plants or insects in the hay bale. This procedure can be costly and can be subjected to human errors when large shipments are involved. Shipments of compressed baled hay are rejected and shipped back to the ports of origin when found to contain host plants or contaminated with Hessian fly resulting in serious economic loss to the hay exporting industry.

Hessian fly is a pest of economic concern to the Japanese government because it destroys cereal plants. The Japanese grow cereal crops including rice, wheat, and barley. The cereal crops grown in Japan are free of Hessian fly infestation, and its introduction may lead to the destruction of cereal crops and economic loss to the country. Buntin and Raymer (1989) investigated the effect of Hessian fly damage on the forage production of susceptible and resistant cultivars of soft red winter wheat and indicated that the total dry matter yield of the forage was reduced by 14 to 46%. Buntin (1999) reported that soft red winter wheat grain yield loss increased linearly with increasing percentage of autumn-infested tillers and spring-infested stems.

Green forages, consisting of various species of grasses and legume crops, are grown and processed into various products such as pellets, cubes, and hay bales. They are very important sources of nutrients in animal feed. Haymaking industries are evolving in Canada where green forages are processed into compressed hay bales for export. There has been a consistent increase in the exportation of hay bales from Canada to Japan. From 1997 to 1999, hay bales exported from Canada to Japan

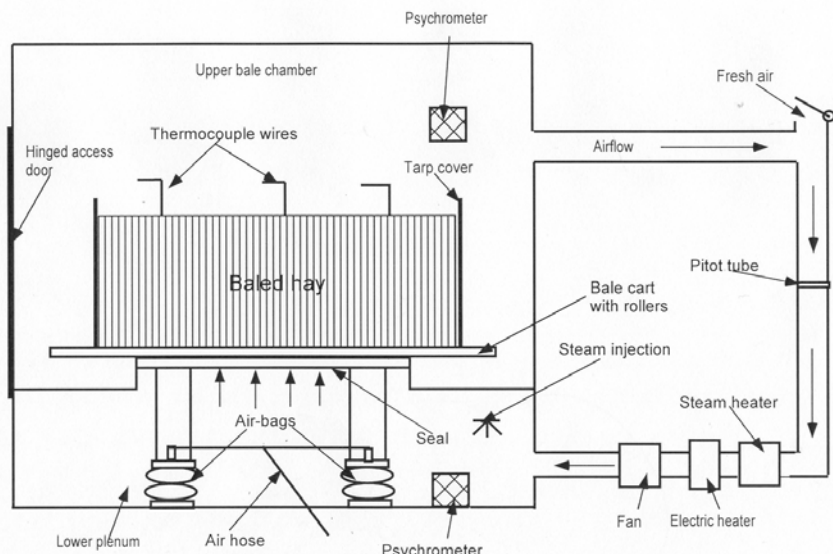


Fig. 1. Schematic diagram of the laboratory heat treatment unit with baled hay in the treatment chamber.

increased by 50% (Dey 2001). Forage exports in 1999 were worth about \$129 million dollars (Agriculture and Agri-Food Canada 2001). There is the potential to increase hay exports to Japan when a phytosanitary quarantine protocol is established between the two countries.

Chemical fumigation, bale compression, and heat treatment have been employed by researchers to disinfest hay bales of Hessian fly puparia. Yokoyama et al. (1993a, 1993b, 1996, 1999) explored the possibility of disinfesting baled hay using chemical fumigation and mechanical bale compression. They indicated that a combined treatment of fumigation and bale compression was effective, and the complete elimination of the Hessian fly puparia was achieved.

Thermal treatment provides an environmentally friendly method of disinfesting agricultural produce (Gaffney and Armstrong 1990; Sharp et al. 1991; Tsang and Fujii 1992; Corcoran et al. 1993; Mangan and Ingle 1994; Neven et al. 1996; Obenland et al. 1999).

Time-temperature mortality tests conducted on the Hessian fly puparia placed in an aluminum block indicated that an exposure of the puparia to air temperature of 60°C for a minimum of 3 minutes resulted in a complete mortality (Sokhansanj et al. 1990; 1992). They indicated that there were survivors at and below a temperature of 52.5°C for an exposure time of 3 min. At a temperature of 47.5°C, some insects survived when exposed for 26 min, but they were killed when the exposure time was extended to 37 min. A temperature of 60°C and an exposure time of 3 min were recommended to ensure a complete mortality of Hessian fly.

Experimental studies were conducted on chopped hay mixed with Hessian fly infested wheat seedlings in a laboratory rotary drum machine. The thermal treatment resulted in the total mortality of the puparial stage of the insect when they were exposed to air temperature of 60°C for 3 min (Sokhansanj et al. 1993).

Sokhansanj et al. (1997) investigated methods of heat treatment of hay bales for the eradication of Hessian fly puparia

in the laboratory. A follow up field test using a commercial baled hay dryer was not successful in completely eliminating the Hessian fly puparia (Sokhansanj 1998). He reported that heat penetration into baled hay was often incomplete mainly due to bulk density and moisture variations within the bale. For the laboratory tests, the bales had to be loosened manually to achieve complete penetration of heat into the bale. For the field tests on bales “as-is”, it was speculated that inadequate heat penetration into the bale during heat treatment resulted in the failure to achieve total mortality of the puparia.

A series of heat penetration studies were conducted on hay bales (mixture of alfalfa and brome-grass) in a laboratory heat treatment chamber using heated air (Opoku et al. 2001). Bales of varying density and moisture content were used. These tests indicated that to reduce the heating time, the bales should be prevented from drying by increasing the relative humidity of the air. The fastest heating time was 5 min and it was achieved at an air temperature of 76°C, relative humidity of 50%, and velocity of 0.33 m/s.

As a follow up to the laboratory experiments conducted on the hay bales at different bale and air conditions, a decision was made to test the mortality of Hessian fly based on the previous laboratory test results. The tests were to be conducted using a higher relative humidity between 40 to 55% in contrast to the field test conducted by Sokhansanj (1998) which used lower values between 2 to 5%.

The objectives of the experiment were:

1. to evaluate the effects of air velocity and relative humidity, and bale moisture content and density on the time required to heat all parts of rectangular brome-alfalfa hay bales to 60°C, and
2. to test the efficacy of heat treatment on Hessian fly puparia in rectangular brome-alfalfa hay bales.

MATERIALS and METHODS

Heat treatment unit

Figure 1 shows a schematic diagram of the laboratory heat treatment unit that was used for the thermal destruction of Hessian fly puparia in the infested wheat seedlings. The unit consisted of a sealed chamber, a centrifugal fan, steam heat exchanger, steam injection system, instrumentation, and data acquisition system. The steam heat exchanger and the steam injection system were manually controlled by adjusting the steam supply valves to control the inlet air temperature and the relative humidity. The centrifugal fan was electrically controlled using a Tosvert-130G2+ transistor inverter (Model VT130G2+2220, Toshiba International Corporation, Houston, TX).

The treatment chamber, measuring 1.78 m long, 0.83 m wide and 1.8 m high, was constructed from 12.7 mm thick plywood reinforced with steel frame. The chamber was insulated with 25.4 mm thick styrofoam sheets. The seams were sealed with

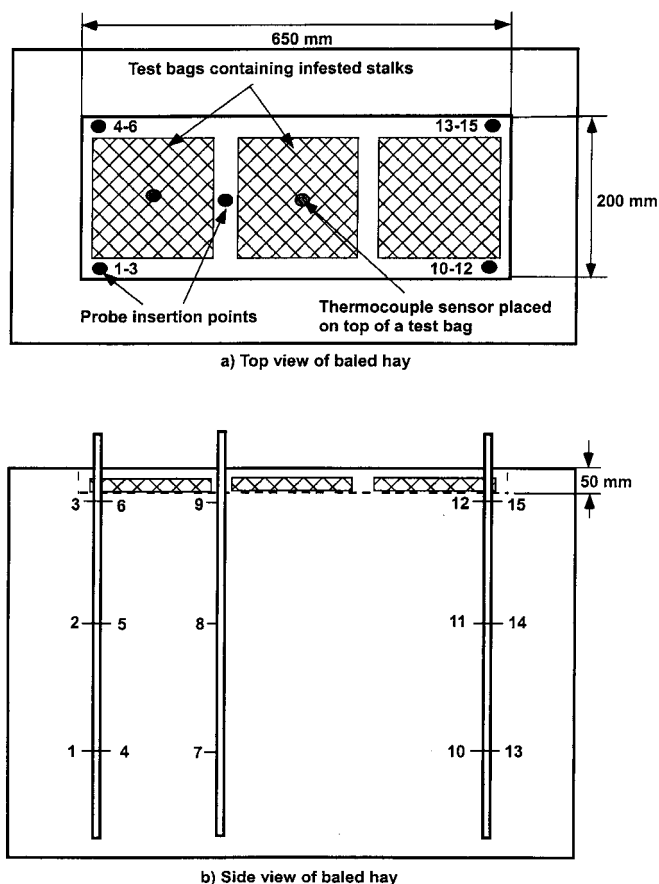


Fig. 2. Placement of test bags and the location of temperature probes in the bale.

silicone sealant. The chamber was divided into a lower plenum and an upper bale chamber. The dividing platform had a 1.02 m by 0.51 m opening in the center. A rolling cart consisting of a 1.21 m by 0.82 m plywood board with 0.66 m by 0.25 m opening at the center was used to transfer the instrumented test bale into the bale chamber. A ratchet and locking tie-down straps were used to tie a tarpaulin around the sides of a test bale to reduce air leakage around the bale.

A variable-speed centrifugal fan capable of delivering air at a maximum volume airflow rate of 0.91 m³/s and at a static pressure of 4.49 kPa was used to circulate heated air through the bale and the chamber. The air was heated in a steam heat exchanger. Additional heat was provided by a 6 kW electric heater (Model TDH-6C, Chromalox, Rexdale, ON). Steam was added to the air at the lower plenum to increase the relative humidity of the incoming heated air.

Instrumentation

Type T copper-constantan thermocouples were used to sense the temperature in the lower plenum, the upper bale chamber, and in the air circulation duct before and after the heaters. All the type T thermocouples were calibrated using a standard mercury in glass thermometer. Two motorized aspirated psychrometers were constructed from type T thermocouples, cotton wicks, fans, and water storage containers. One was installed in the lower plenum and the other in the upper bale chamber to measure the wet and dry bulb temperatures. Relative humidity was measured to $\pm 3\%$.

Three type T thermocouples were attached to a 3.2 mm diameter fiberglass rod with sensor tips spaced 120 mm apart. A heat shrink tube secured the thermocouple sensors to the rod. Five rod assemblies were prepared. Figure 2 shows the temperature probes insertion points to determine the temperature of the lower, middle, and upper parts of the bale.

Pressure taps were placed in the lower plenum and in the upper bale chamber to measure the air pressure drop across the bale. Flexible plastic tubings, with internal diameter of 3.2 mm, connected the taps to a digital manometer (Model HHP-100A, Omega Engineering, Inc., Stamford, CT.) to record the pressure drop across the bale. A calibrated averaging pitot tube assembly measured the average speed of air through the circulating ducts. A data logging unit, Sciometric Model 8082A, (Sciometric Instruments Inc., Manotick, ON) was used to receive all the output signals from the sensors. The data were displayed on a monitor during a test and stored on the hard drive disk for further analysis.

Test material preparation and procedure

Hay Brome-alfalfa hay bales were obtained from a hay shed at the University of Saskatchewan. Eight good quality bales were selected from inside the hay stockpile about three layers from the top. The hay bales were grouped as low (113 and 116 kg/m³), medium (120 and 124 kg/m³) and high density (144, 145 and 148 kg/m³). A calibrated Delmhorst, Model HTM-2, (Delmhorst Instrument Co., Towaco, NJ) bale moisture sensor was used to assess the initial moisture content of the bales. The bales were randomly selected for testing.

Cavities, having approximate dimensions of 650 mm long, 200 mm wide and 50 mm deep, were made on top of each bale to accommodate the test bags containing the infested wheat seedlings. A sample of about 100 g was removed from inside the cavities on top of the bale for moisture measurement. Each bale was weighed and its dimensions (length, width, height) were measured. After the heat treatment, the bale was weighed and another set of hay sample was taken for moisture measurement. Moisture contents were measured by the oven method according to the ASAE Standard S358.2 (ASAE 1998).

Test insects Young wheat seedlings infested with the puparial stage of the Hessian fly were obtained from the Southern Crop Protection and Food Research Centre of Agriculture and Agri-Food Canada (AAFC) in London, Ontario. Each wheat seedling was infested with an average of 3.26 Hessian fly puparia based on laboratory control. A total of 24 polyester mesh bags containing an average of 125 infested wheat seedlings per bag were prepared. Before bagging and packaging, the wheat seedlings were allowed to air-dry for 3 hours to remove excess moisture in the mesh bags. The mesh bags containing the infested wheat seedlings were packed in a styrofoam container with cold gel pads and shipped from London, Ontario to the University of Saskatchewan in Saskatoon, Saskatchewan. Upon receipt, the mesh bags were stored in a cold storage room at a temperature of 5°C and relative humidity of 85% to prevent the dessication and mortality of the puparia (Foster et al. 1988; Yokoyama et al. 1996). The temperature and relative humidity inside the package were not measured.

Heating method To carry out a test, a bale was placed over a cart. The bale's cut edge (355 x 914 mm) was oriented toward the incoming heated air. Three labeled mesh bags containing the

Table 1. Initial conditions of baled hay used in the heat treatment tests.

Test #	1	2	3	4	5	6	7	Mean	S.D.
Length (mm)	991	1042	988	987	1057	970	994	1004	32
Width (mm)	363	364	358	368	364	368	360	364	4
Height (mm)	466	477	457	461	467	450	461	463	8
Initial mass (kg)	19.5	22.6	23.4	19.0	25.8	19.3	24.5	22.0	2.8
Initial M.C. (% w.b.)	9.8	9.7	9.9	8.5	9.9	9.8	9.1	9.5	0.5
Density (kg/m ³)	116a*	124a	145b	113a	144b	120a	148b	130	15

*Values within the same row followed by the same letter are not significantly different at 0.05 level.

infested wheat seedlings were removed from the cold room and immediately placed inside the cavity on top of the bale and covered lightly with the hay material. Five temperature probes were inserted into the bale and another two thermocouple sensors were placed on top of the test bags. Figure 2 shows the placement of mesh bags, temperature probe insertion and temperature sensing points within the bale.

The treatment chamber was pre-heated to an air temperature of 67°C or above and steam was introduced to increase the relative humidity. Once equilibrium condition was reached, the chamber door was opened and the artificially infested bale was rolled into the chamber.

Temperature data were recorded at 10 s intervals until all parts of the bale reached 60°C. The target temperature was maintained for three minutes before the steam supply was turned off. The bale was cooled to represent the standard practices in the compressed hay producing industries to preserve quality. The bale was then removed from the chamber. The mesh bags containing the Hessian fly puparia were immediately removed from the bale and stored inside the styrofoam container in the cold room. Two speed settings (35 and 40 Hz), on the inverter controlling the fan, were used in the experiments. Air velocity through the bale was estimated using the VanDuyne and Kjølgaard (1964) equation given as:

$$\frac{dP}{dL} = 0.072B^{2.31}V^{1.60} \quad (1)$$

where:

- dP = pressure drop (Pa),
- dL = length of air travel (m),
- B = dry matter density (kg/m³), and
- V = air velocity (m/s).

The tests were conducted in a completely random order. The bulk density and air velocity were varied. The air temperature, relative humidity and air velocity reading were averaged over each heating cycle. One-way analysis of variance (ANOVA) and Scheffe multiple comparisons (SPSS for Windows, Release 9.0, SPSS Inc., Chicago, IL) were used to test for significance of the variables used in the laboratory heat disinfestation tests. Yokoyama et al. (1993b) analyzed the data for the response of Hessian fly to bale compression using a similar statistical analysis to test for the overall significance of four sizes of puparia, five positions in each bale, three sections in each bale, six species with controls and two compressor types. Three mesh bags were not heat-treated and were used as controls. They were placed on a bale and ambient air (20 – 27°C) was circulated through them.

Post treatment emergence

After the tests were completed, the treated mesh bags containing the Hessian fly puparia including the controls were shipped back to AAFC in London, Ontario for a standard 75-day post treatment emergence. The wheat seedlings were set up in separate cages on August 1, 2000. Acetate cages, 380 mm in diameter and 450 mm long, having a cloth sleeve access at one end and four screened ventilation vents on the opposite end were set up with paper towel spread at the bottom. The contents of the three test bags (from one bale) were removed and distributed over the paper towel. The set-up was sprayed with deionized water until wet. They were then covered with more paper towel and a plastic sheet to inhibit drying. Water was added as needed to prevent drying of the wheat stalks and the puparia. The contents of three mesh bags used from each test were placed in one cage. In all, eight cages were set up to accommodate the contents of the mesh bags from the seven heat treatments and the control. The conditions in the cages were maintained at 21°C with 70% relative humidity for 16-hour photophase until emergence had ceased.

RESULTS

Initial bale conditions

Table 1 lists the bale dimensions, initial mass, moisture content, and density of the seven bales that were used in the thermal treatment tests. The dimensions of the bales ranged from 970 to 1057 mm for the length, from 360 to 368 mm for the width, and from 450 to 467 mm for the height. The initial mass ranged between 19.0 and 25.8 kg, and initial moisture content varied between 8.5 and 9.9% wet basis. The bale density ranged between 113 and 148 kg/m³. Statistical analysis indicated there was a significant difference in the bulk density of the bales used in the heat disinfestation tests ($P < 0.05$). However, the bale bulk density did not significantly affect the heating time. The low variability in the initial moisture content (S.D. = 0.5%, w.b.) of the bales indicated their state of equilibrium with the environment. The variability in the moisture content was probably caused by the location of the bales in the stack.

Velocity, temperature, relative humidity, and heating time

Thermal disinfestation treatment conditions and the heating times are summarized in Table 2. The minimum air temperatures used in the heat treatment ranged from a low of 67°C to a high of 70°C. The maximum air temperatures reached at the end of the heating cycles varied between 77 and 80°C. Inlet air temperature did not significantly effect the heating time in these experiments.

Table 2. Treatment conditions in the chamber during the heating cycle.

Test #	1	2	3	4	5	6	7
Maximum air temperature (°C)	78	77	79	78	80	79	78
Minimum air temperature (°C)	67	68	68	69	70	68	68
Average air temperature (°C)	73	74	75	75	75	76	74
Maximum air relative humidity (%)	58	46	45	52	48	42	45
Minimum air relative humidity (%)	34	24	25	25	26	21	22
Average air relative humidity	53a*	42b	41b	43b	43b	39b	41b
Bale temperature difference *(°C)	11	15	18	15	16	15	17
Inverter speed setting (Hz)	40	35	40	35	35	40	35
Air velocity through bale (m/s)	0.30a	0.29a	0.29a	0.28a	0.20b	0.31a	0.23b
Bale heating time (min)	8a	14a	26b	15a	12a	23b	20b

* Values within the same row followed by the same letter are not significantly different at 0.05 level.

** Temperature difference between the fastest and the slowest heating points within the bale at the end of the heating cycle

The minimum air relative humidity during the heating process varied between 21 and 34%; the maximum values varied between 42 and 58%. Similar to air temperatures, higher air relative humidities were generally recorded at the end of the heating cycle. Air relative humidity had a significant effect on the heating time ($P < 0.05$) in the disinfestation tests. Table 2 shows higher relative humidity (53%) resulted in a shorter heating time.

Two velocity settings were used in the laboratory experiments and the estimated air velocities through the bales ranged from 0.20 to 0.31 m/s. The low air velocities estimated for tests #5 and #7 were expected since the tests were run at a low velocity setting and the bales' bulk densities were high (Table 2). The high air velocities estimated for tests #2 and #4 were probably due to the bales' low bulk densities. Air velocities did not have any significant affect on the heating time.

The shortest heating time was 8 min and this could probably be attributed to the high relative humidity (53%). The longest heating time was 26 min and this was probably due to the lower relative humidity (41%). The coefficient of determination (R^2) between the bale heating time and air relative humidity ($R^2 = 0.59$) was high compared to the coefficient of determination between the heating time and the other factors such as air temperature ($R^2 = 0.397$), air velocity ($R^2 = 0.038$) and bulk density ($R^2 = 0.168$). The lower relative humidity values obtained might be due to the lower ambient air relative humidity during the tests and inadequate steam flow from the steam supply lines.

Figure 3 shows typical temperatures and relative humidities during warm-up, heating, and cooling cycles. The warming of the chamber took about 30 minutes. The temperature at location 7 (see Fig. 2) asymptotically approached the inlet temperature and the temperatures at location 8, 9, and on top of the mesh bag almost stayed close together up to the end of the heating phase. The temperature on top of the mesh bag was slightly higher than the temperatures at locations 8 and 9. This could probably be due to its exposure to a higher surrounding temperature on top of the heating chamber as indicated by the outlet temperature. The thermocouple at location 9 was close to the mesh bags containing the Hessian fly puparia. The temperature probes were located one above the other vertically at the center of the bale with the probe at location 7 closer to the inlet temperature.

At the end of the heating phase, the relative humidities and the temperatures began to drop rapidly when the steam injection was stopped and ambient room air (between 20 and 27°C) was introduced to begin the cooling phase.

Table 2 shows the temperature difference between the fastest and the slowest heating locations within the bale at the end of the heating cycle. The temperature difference ranged from 11 to 18°C. The shorter the heating time for the slowest heating point to reach a temperature of 60°C, the smaller the temperature difference. The temperature difference can be reduced if a bale heating time is decreased by increasing the inlet air relative humidity, air velocity, and air temperature (Opoku et al. 2001).

Hessian fly puparia mortality

The estimated total number of Hessian fly puparia that were used in the heating tests and control was 9780. The number of puparia used as control was 1220. For each disinfestation heating test, 1220 puparia were used. Table 3 shows the emergence of Hessian fly insects after the cages have been set up. A total of 241 flies emerged in the control cage. This represented approximately 20% of the total amount of puparia used as controls. Yokoyama et al. (1993a) reported that 17% of the Hessian fly puparia survived when they were used in field controls. Yokoyama et al. (1993b) again reported a survival range of 19 to 58.3% for Hessian fly puparia used as controls.

Table 3. Emergence of Hessian fly insects used in the heat treatment and control during incubation.

Test runs and control	Total number of emergence	Emergence (%)
#1	0	0
#2	0	0
#3	0	0
#4	0	0
#5	0	0
#6	0	0
#7	0	0
Control	241	20

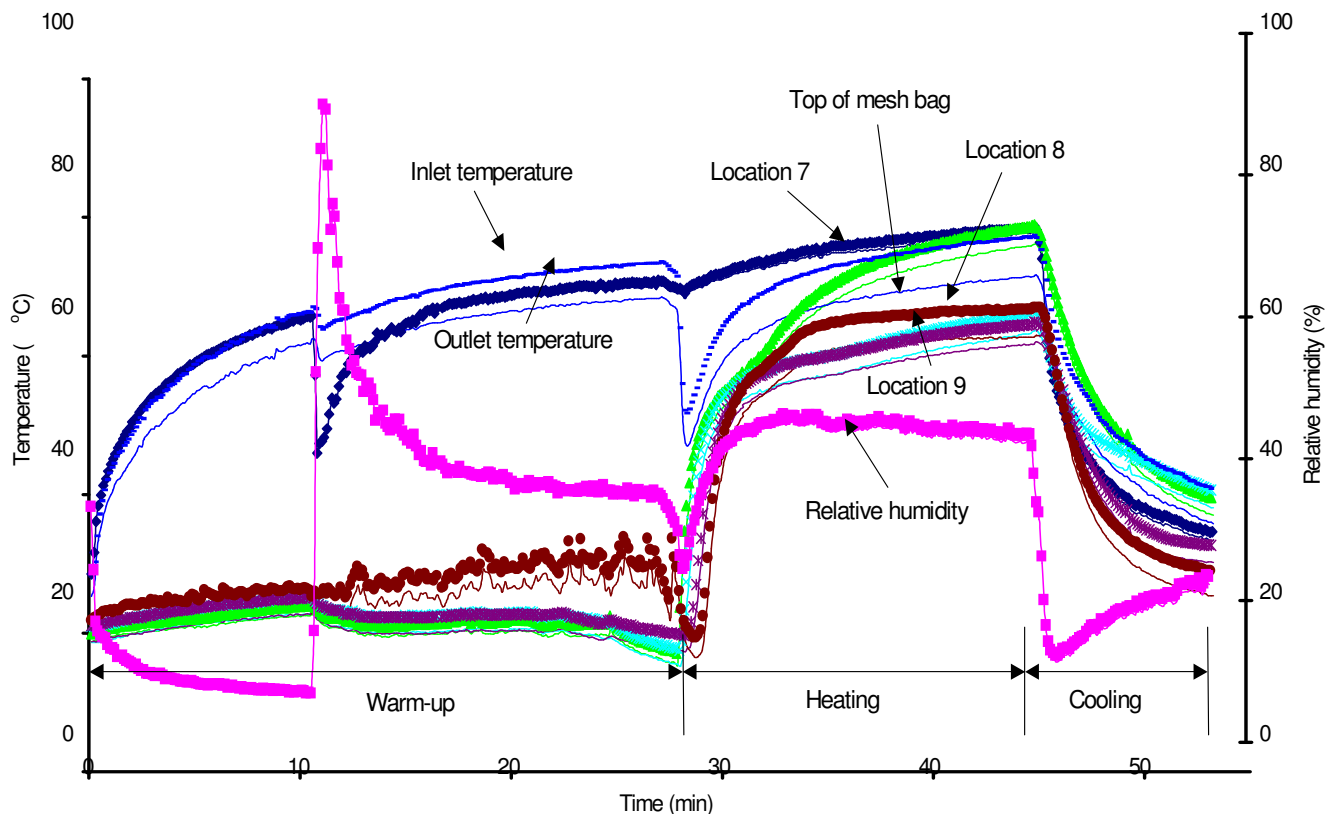


Fig. 3. Typical recorded temperatures and relative humidity during warm-up, heating, and cooling cycles.

There was no emergence from any of the mesh bags placed in the heat-treated bales. The heat treatment resulted in a total mortality of the Hessian fly puparia. The Hessian fly puparia were killed at all the initial air and bale conditions used in the laboratory experiments. To ensure a total kill of the Hessian fly, higher air relative humidity $\geq 53\%$, combined with a higher air temperature $\geq 76^\circ\text{C}$ and air velocity ≥ 0.31 m/s for a minimum of 20 min, should be used. However a pilot scale treatment unit should be set up to study the effects of air temperature, relative humidity, and air velocity on bales artificially infested with Hessian fly in order to optimize the treatment schedule.

The number of flies that emerged from the control was lower than expected. Based upon the number of estimated puparia per seedling close to 1000 flies were expected to emerge in the control cage. The low emergence could probably be due to lower starting population than estimated. Removal of excess moisture from the wheat stalks and the desiccated environment in the package during transport and storage might also have contributed to the low emergence (Foster et al. 1988; Yokoyama et al. 1994).

CONCLUSIONS

As a follow up to our previous laboratory experiments on hay bales, bales of varying properties, artificially infested with Hessian fly, were subjected to varying air conditions to verify the mortality of Hessian fly puparia in a laboratory heat treatment unit. The following conclusions can be drawn from the heat treatment tests:

1. Heating times ranging from 8 to 26 min were required to heat all parts of the brome-alfalfa bales to 60°C and retain the temperature for 3 min in order to ensure the thermal death of the Hessian fly puparia based on the initial bale and air conditions. Air relative humidity had a significant effect on the heating time compared to air temperature, air velocity, and bale bulk density.
2. Total mortality of the Hessian fly puparia was achieved in these laboratory heat disinfestation tests when all parts of the bale were heated to a temperature of 60°C and above, and maintained at this condition for a minimum of 3 min.

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