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Effects of Microwave Radiation on Two Species of Stored-Product Insects.

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INTRODUCTION

For plant quarantine purposes, fumigation treatment is at present considered to be the best method for disinfestation of cereal grains imported from foreign countries. As methyl bromide is the most effective fumigant for insect control, it is widely used for disinfestation. However, since the application of methyl bromide requires relatively long periods and it may form residues when used on foodstuffs, alternative methods of insect control have been developed.

Radiofrequency (RF) energy may be used to control insects infesting stored cereals and cereal products. The amount of energy absorbed by the materials in an RF field is dependent upon the dielectic property of the material, the frequency and intensity of the applied field. Such absorbed energy results in heating which is commonly called "dielectric heating". Microwaves are the electromagnetic waves in the RF region with frequencies higher than about 1,000 MHz, which are more effective in generating dielectric heating than lower wave ranges. The lethal effects of microwave energy are considered to be caused entirely by the heat generated within the materials being irradiated by a microwave energy source.

The effectiveness of microwave treatment for insect control has been reported by BAKER *et al.* (1956), KIRKPATRICK *et al.* (1971), NELSON (1973), WATTERS (1976) and KOBAYASHI *et al.* (1974).

The principle advantage for microwave treatment is total freedom from harmful residues in foodstuff, which may become even more objectionable in the future. Moreover, this type of treatment can kill all stages of insects in grain with only a very short exposure time.

The purpose of our work was to determine the effectiveness of a commercially available microwave oven in controlling stored-product insects and to supplement existing information on the effectiveness of microwave radiation for the control of two species of stored-product insects.

MATERIALS AND METHODS

The equipment consisted of a pulsed magnetron oscillator and a turn-table in a radiofrequency chamber of microwave 21 cm high × 33 cm wide × 33 cm deep. The oscillator frequency was fixed at 2,450 MHz with an average power output of 1.15 KW.

Test species were the confused flour beetle, *Tribolium confusum* JACQUELIN DUVAL and the small rice weevil, *Sitophilus oryzae* LINNÉ. All test insects were obtained from laboratory cultures reared at a temperature of 26±1°C and 70% RH. The ages of the insects tested are indicated in or under each table on the experimental results. The samples of *T. confusum* eggs were obtained by placing ca. 1,400 adults in 1,400 g of wheat flour for a two days oviposition period. Larvae and pupae of *T. confusum* were treated when 32–34 days and 38–40 days old post-oviposition respectively, i.e., larvae were estimated to be almost mature and pupae were estimated to be about 4 days pre-adult emergence. The samples of *S. oryzae* eggs, larvae and pupae were obtained by placing ca. 2,500 adults in 250 g of wheat for a three day oviposition period. The samples of eggs thus obtained were then irradiated immediately. Larvae and pupae of *S. oryzae* were treated at the age of 12–15 days and 22–25 days post-oviposition respectively. These stages in development were almost the same as those of *T. confusum* which were also treated.

Host medium for insects was either one of northern spring wheat, hard winter wheat or red spring wheat.

Moisture content of the wheat or flour used in every experiment was determined by weighing and then heating the flour to 130°C for 2 hours and reweighing. With wheat grain, it was milled first. When necessary, grain was tempered by adding water with agitation and then holding it for 24 hours, to insure a uniform distribution of moisture within the grain.

The temperature at the mid-point of the depth at 1.5 cm in the wheat or flour was monitored just after irradiation with thermocouples and a potentiometer.

Samples of each stage of each species were tested separately. Host medium of *T. confusum* was wheat flour, while whole wheat was used for *S. oryzae*. Samples of the wheat or flour were prepared for microwave treatment by putting 50 g of the host medium in 140 m*l* glass beakers 5.2 cm in diameter and 6.5 cm high. The top of the beaker was covered with the polyethylene film generally used for food wrapping, and fastened with rubber bands.

For each stage of each species which could be counted, 50 test insects were introduced into each beaker. For enclosed forms of *S. oryzae* within the grain and tiny *T. confusum* eggs, infested wheat or flour was thoroughly mixed before it was placed in the beaker. Each test was replicated three times.

The samples were placed on the center of the turn-table in the microwave oven for irradiation. A series of exposure periods was used to ensure a level that caused mortaility. After irradiation, each sample was allowed to cool to room temperature. The samples of adults were stored for 1 day after which mortality was assessed. The samples of all other stages were transferred to paper bags with the irradiated medium and held to permit development to the adult stages, after which counts were made of survivors.

Criterion for death in the adult stage was complete immobility as determined by visual examination while gently blowing over the insects. For those within the grains, the criterion of death was failure to emerge after the normal period in an environment favorable for development. For *T. confusum* eggs and enclosed forms of *S. oryzae*, mortality of each was estimated by using the following formula modified from the one of GARRETT and LANGFORD (1969):

$$\% \text{ mortality} = \frac{\text{emerging check no.} - \text{emerging treated no.}}{\text{emerging check no.}} \times 100$$

In addition to mortality determinations, four experiments were conducted.

1. The rates of heating of wheat at 12.6%, 14.3% and 16.0% m.c. and the mortalities of 50 adults of *S. oryzae* in each medium were determined.

2. The fecundities of *T. confusum* adults, irradiated in the wheat flour (11.5% m.c.) with sublethal exposures of 6, 9, 12, 15 and 18 sec., were determined. The samples of each of 40 surviving adults were deposited in 50 g of wheat flour at 11.5% m.c. for three days and stored until adult emergence ceased. Fecundity percents were calculated with the formula used in the estimation of the mortality of *T. confusum* eggs and the enclosed forms of *S. oryzae*.

3. *T. confusum* adults 2–7, 37–45, 67–71, 103–107 days old were irradiated in wheat flour at 12.3% m.c. to determine their susceptibility at 10, 15, 20, 25, and 30 sec. exposures.

4. The heat distribution in a microwave oven during irradiation was determined by the mortality of samples of 50 adults of *T. confusum* placed separately at the upper, middle, and lower levels in a vertical direction and/or at the center, middle, and edge of the turn-table in a lateral direction.

All experiments were conducted in a room 26±1° C and 70% RH.

To determine the 95% lethal temperature (LT–95), the probit method suggested by BLISS (1935) and KONO (1951) was used, and probit transformation from percent mortality was used in the factor analysis of lethal effectiveness such as mositure content of host medium, adult age, and position in the oven except in the case of vertical distribution. In the factor analysis of the fecundities of *T. confusum* adults, results were analzyed by transformating the emergence number of adults to the logarithmic scale, and by analysis of variance and mean separation by Duncan's multiple range test (LECLERG, 1957; DUNCAN, 1955).

RESULTS AND DISCUSSION

A comparison of susceptibility of the two test species at their different stages of development to microwave, medium temperatures for 95% insect mortality (LT–95) and their estimated exposured periods are summarized in Table 1. For obtaining 95% insect mortality, 25.0 sec. exposured period was necessary to the *T. confusum* and 23.5 sec. to the *S. oryzae*. Based on LT–95 for adults, *S. oryzae* was more susceptible than *T. confusum*. This was in agreement with the data of WHITNEY *et al.* (1961) and NELSON and KONTACK (1966). With *T. confusum* treated in wheat flour, larvae are significantly much more susceptible ($P<0.05$) than the other stages, however, between the remaining stages there was no significant difference. With *S. oryzae*, the egg stage was seemed to be more susceptible than the other stages, however, the LT–95 of all stages were almost the same ($P>0.05$). These results were not in accordance with the data of BAKER *et al.* (1956) who found that, when treated in whole wheat flour at 2,450 MHz, *T. confusum* adults were more susceptible than either the larval or egg stages. WHITNEY *et al.* (1961),

TABLE 1. 95% lethal temperature (LT–95) produced in host media by microwave treatment to all stages of *T. confusum* and *S. oryzae* at the estimated exposured periods

Species	Stage	Age (days)	Moisture content of medium (%)	LT–95 (°C)	The estimated exposured periods for LT–95 (sec.)
T. confusum	adult	28-35	11.8	66.5 a*	23.5
	pupal	38-40	〃	65.4 a	25.0
	larval	32-34	〃	53.0 b	18.0
	egg	0-2	〃	65.6 a	23.0
S. oryzae	adult	49-56	12.1	59.7 c	23.5
	pupal	22-25	13.4	60.3 c	21.5
	larval	12-15	12.9	58.4 c	20.0
	egg	0-3	12.8	56.7 c	19.5

* Different letters indicate differences at 5% level of probability.

NELSON *et al.* (1966) and NELSON (1973) described that in general, the adult insects were more susceptible to radiofrequency (RF) exposure than the immature stages, especially species such as *S. oryzae* and *S. granarius*, where the immature stages develop within the kernel. They attributed the difference in susceptibility between the adult and the immature stages to the partial shielding of immature forms. Early studies by PYENSON (1933) showed that certain materials surrounding insects tend to shield them when exposed to high frequency fields, thereby insects inside kernels of grain may be partially shielded. However, according to the principle of control of insects by microwave radiation, insects exposed to microwave experience a rapid rise in temperature due to internal generated heating, as insects absorb energy from the radiofrequency field and lethal effects of such exposures are generally attributed to lethal temperature arising from dielectric heating. From this point of view, it seems reasonable that enclosed forms ought to be exposed much more to heating than exposed forms, subsequently mortalities of the immature stages would be higher than the adults. The data reported here seems to support our concept.

T. confusum adults that survived sublethal exposures at 2,450 MHz were capable of reproduction (Table 2). The rate of reproduction was only a bit lower even though

TABLE 2. Fertility of the adults* of *T. confusum* with microwave treatment

Exposured period (sec.)	Mortality %	Emergence no. of adults	Fecundity %
0	0	396.0	100.0 a**
6	0	489.7	123.7 b
9	0	503.3	127.1 b
12	4.7	380.7	96.1 a
15	64.9	360.7	91.1 a
18	87.8	326.7	82.5 a

* Adults treated were 36–44 days old.
** Different letters indicate differences at 5% level of probability.

adults exposed to microwave treatment suffered 87.8% mortality. WHITNEY *et al.* (1961) reproted that *S. oryzae* and *T. confusum* survived sublethal exposures of 39 MHz were capable of reproduction. WEBBER *et al.* (1946) also reported reproductive capability of *T. confusum* that survived exposures in flour at 11 MHz. It is very interesting that by 6 or 9 sec. exposures of microwave, emergence of adult numbers was more than the control (Table 2). It appears that a very slight stimulus of microwave treatment gives the adults an increased reproductive capability.

Influence of insect age on the effects of microwave treatment was studied in an experiment where four groups of *T. confusum* adults 2–7, 37–45, 67–71, 103–107 days old were subjected to identical microwave exposures in wheat flour. It was found that 2-7-day-old adults were significantly less susceptible than the other older age groups (Table 3). These results are a bit different from the experimental data of WATTERS (1976) and NELSON and KANTACK (1966) who reported there was no difference in mortality between adults of different ages.

The heating rates of wheat samples at 12.3%, 14.3% and 16.0 % m.c. were found to be similar (57°C per 30 sec.) (FIG. 1). Temperature in the host medium tended to rise

TABLE 3. 95% lethal temperature of the wheat flour due to microwave treatment of the different ages of *T. confusum* adults at 12.3% moisture content

Age (days)	LT-95(°C)
2-7	76.1 a*
37-45	66.6 b
67-71	66.2 b
103-107	66.0 b

* Different letters indicate differences at 5% level of probability.

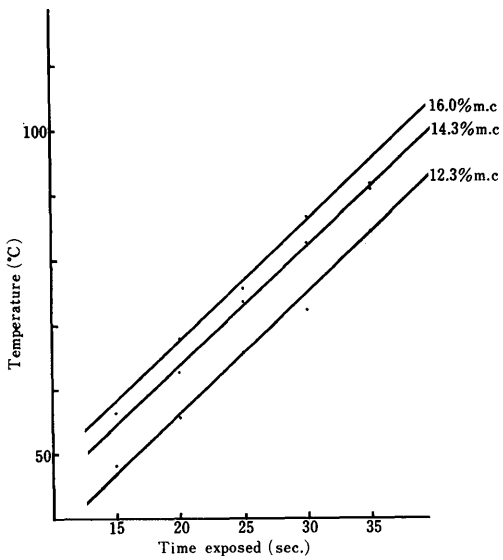

FIG. 1. Rate of heating of wheat at 12.3%, 14.3% and 16.0% m.c. irradiated at 2,450 MHz.

TABLE 4. Percent mortality of *S. oryzae* adults* in whole wheat at 12.3%, 14.3% and 16.0% moisture content irradiated with microwaves at indicated exposure periods

Exposured period (sec.)	Mortality %		
	m.c. 12.3% a**	14.3% a	16.0% a
15	54.4	51.7	57.8
20	77.3	88.3	83.4
25	99.3	99.3	97.3
30	100.0	100.0	100.0
35	100.0	100.0	100.0

* Adults treated were 25–32 days old.
** Same letter indicates non difference at 5% level probability.

significantly with the increase in moisture content ($P<0.01$), however within the range of these moisture contents, mortalities of *S. oryzae* adults were not affected significantly ($P>0.05$) (Table 4). NELSON and KONTACK (1966) reported that within the moisture range trom 12% to 16%, RF treatment (39 MHz) may be slightly more effective as the moisture content of the wheat is increased. WATTERS (1976) also stated that an increase in the moisture content of wheat resulted in a temperature increase in the grain subsequent to the higher mortalities of *T. confusum* adults. However, adults had less close contact to the wheat kernels than to the wheat flour. Therefore it seems reasonable to expect that differences in moisture content of wheat kernels did not result in any distinguishable

TABLE 5. Percent mortalities of *T. confusum* adults* in the beakers placed at each of three indicated levels in the oven

Exposured period (sec.)	Mortality %		
	1 cm lower a**	6 cm middle b	11 cm upper a
15	70.7	24.7	62.0
20	87.3	30.0	92.0
25	100.0	48.7	99.4
30	100.0	66.0	100.0
35	100.0	99.3	100.0

* Adults treated were 10–21 days old.
** Different letters indicate difference at 5% level probability.

TABLE 6. Percent mortalities of *T. confusum* adults* and host media temperatures in the beakers placed at each of three indicated positions in the lateral directions within the oven

Exposured period (sec.)	Mortality percent and temperature					
	0 cm center a**		6 cm middle a		12 cm edge a	
20	39.3%	45.0°C	56.5%	44.7°C	33.3%	44.3°C
25	89.0	52.7	66.1	50.7	63.3	50.0
30	100.0	58.3	89.3	58.0	96.7	56.7
35	100.0	64.3	100.0	60.7	100.0	61.3

* Adults treated were 28–39 days old.
** Same letter indicates non difference in mortality pescent at 5% level probability.

differences in the mortalities of *S. oryzae* due to microwave treatment.

Differences in mortality due to the location of insects in the oven was studied as indicated in Table 5 on vertical effects and Table 6 on lateral effects. Adults mortality in the vertical direction was significantly lower ($P<0.01$) in the middle part (6 cm high from the turn-table) than at the other two levels, yet there was no significant difference in the lateral directions. Temperature increases were significantly higher ($P<0.05$) at the center of the table than the middle part and the edge (Table 6). Consequently, material to be disinfested should be placed at the same level on the turn-table in the oven.

SUMMARY

1) The susceptibility of *Tribolium confusum* JACQUELIN DUVAL and *Sitophilus oryzae* LINNÉ to microwave energy was determined by irradiating beakers of infested wheat or flour with a pulsed magnetron oscillator frequency of 2,450 MHz.

2) For obtaining 95% insect mortality, 25.0 sec. exposured period was necessary to *T. confusum* and 23.5 sec. to *S. oryzae*. Based on adult mortality, *S. oryzae* was more susceptible than *T. confusum.*

3) Susceptibility of larval stages of *T. confusum* was significantly higher than the other stages, between which there were no significant differences. All stages of *S. oryzae* had no significant differences in their susceptibility to microwave treatments.

4) *T. confusum* adults surviving exposures of microwave were capable of reproduction, although at a somewhat lower rate.

5) Two-to-7-day-old adults of *T. confusum* were significantly more susceptible than older adults.

6) Temperatures in the host medium rose in proportion to the moisture content which increased within the range from 12.3% to 16.0% at the same exposured period, while *S. oryzae* adult mortalities were not proportional.

7) *T. confusum* adult mortalities due to the location of the beaker in the oven was significantly lowered at the middle level part in the vertical direction, yet was not significantly different in the lateral direction.

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摘　　要

2種類の貯蔵穀物害虫に対するマイクロ波照射の影響

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1. マイクロ波照射によるヒラタコクヌストモドキ (*Tribolium confusum* JACQUELIN DUVAL) およびココクゾウ (*Sitophilus oryzae* LINNÉ) 各態の感受性および成虫の令による感受性の相異，照射後の生存虫の産卵能力，被照射小麦の含水率の差異による照射効果，マイクロ波発生装置（電子レンジ）内の被照射物の位置の相異による効果のそれぞれについて調査した。

オーブン内にターンテーブルのある市販電子レンジにに害虫の付着した小麦粒，あるいは小麦粉をそれぞれ 50 g づつ，140 ml 容ガラスビーカーに入れ，所定時間，マイクロ波 (2,450 MHz) を照射した。致死率はプロビット解析によって 95% 致死温度を出し，さらに致死率をプロビット変換して要因解析を行なった。

2. ヒラタコクタヌストモドキの全ステージの 95% 致死を得るためには，25.0 秒のマイクロ波照射が必要であり，ココクゾウに対しては 23.5 秒の照射が必要であることが明らかとなった。

成虫の致死効果によれば，ココクゾウはヒラタコクヌストモドキよりマイクロ波に対する感受性が高い。

3. ヒラタコクヌストモドキの幼虫は他のステージより感受性が高いが，成虫，蛹，卵の間には有意差がなかった。

ココクゾウの場合，各態間の感受性の有意差は見い出せなかった。

4. マイクロ波のヒラタコクヌストモドキに対する18秒照射で 87.8% の致死率があったが，生存虫の産卵率はコントロールに比べ，82.5% を有し，若干おちただけであった。

5. ヒラタコクヌストモドキの成虫の令による感受性は，羽化後 2～7 日目の成虫が，これより老いた成虫より感受性が若干低かった。

6. 穀粒の含水率を 12.3%，14.3%，16.0% とかえて含水率の相異によるココクゾウの致死率の変化を調査したが，殺虫率に有意差を認めなかった。

7. オーブン内の位置によるヒラタコクヌストモドキ成虫の殺虫率は，垂直方向の中間部で有意に低かったが，水平方向での有意差は認められなかった。