Journal of Economic Entomology Advance Access published March 22, 2015

Commodity Treatment and Quarantine Entomology

Ethyl Formate Fumigation of Dry and Semidry Date Fruits: Experimental Kinetics, Modeling, and Lethal Effect on Carob Moth

HAITHEM BESSI,[1,2,3] SIHEM BELLAGHA,[1,2] KAOUTHAR GRISSA LEBDI,[4] VERONIQUE BIKOBA,[5] and ELIZABETH J. MITCHAM[5]

J. Econ. Entomol. 1–7 (2015); DOI: 10.1093/jee/tov032

ABSTRACT Ethyl formate (EF) was studied as a fumigant agent with the objective to replace methyl bromide (MB) for date fruit disinfestations. Date fruits *Phoenix dactylifera* 'Deglet Nour' with different initial moisture content (16% for dry dates, 20% for semidry dates, and a mixture of the two types) were separately fumigated with EF at different concentrations: 28.6, 57.3, 85.9, and 114.6 g/m^3 for 2 h. Experimental data of EF sorption during fumigation was successfully fitted to Peleg's model. This model allows the prediction of the effects of date moisture content and EF concentration on sorption behavior. Samples with different moisture content showed similar EF sorption behavior. Dates were artificially infested with carob moth (*Ectomyelois ceratoniae* (Zeller)) at different life stages. Eggs, third- and fifth-instars, and pupae were exposed to 28.6, 57.3, 85.9, and 114.6 g/m^3 EF for 2 h. Among these life stages, fifth-instars were the most resistant to EF fumigation. A 2-h fumigation with 114.6 g/m^{3}EF provided complete control of eggs, third-instars, and pupae of carob moth, and generated 91.6% mortality of fifth-instars. A longer fumigation time or higher EF concentration may provide complete control of all life stages of carob moth.

KEY WORDS carbon dioxide, moisture content, Peleg model, sorption, Vapormate

The world production of dates has increased from about 2.8 million tons in 1985 to 6.9 million tons in 2005 (Food and Agriculture Organization of the United Nations [FAO] 2005). Date fruit *Phoenix dactylifera* ('Deglet Nour'), which has the highest economic value in countries such as Tunisia, Algeria, Israel, and the United States, may be attacked by serious pests, especially the carob moth (*Ectomyelois ceratoniae* (Zeller) (Pyralidae: Lepidotera)). This polyphagous insect is able to infest 49 host plant species worldwide. The major host plants are carob, orange, pomegranate, and dates (Doumandji 1981). Depending on climatic conditions and host plants, carob moth may produce up to four generations per year (Doumandji 1981, Wertheimer 1958). Date maturity stages have been classified as "Hababouk," which is the earliest stage of fruit development; "Khimri," characterized by a rapid weight increase and high water content; "Khalal," where sucrose content reaches higher levels and water content is reduced; "Rutab," which have high levels of monosaccharides with a softening texture; and the final maturity stage "Tamar," where the final sugar content is reached with the lowest moisture content (Djerbi 1994). Idder et al. (2009) found that adult female carob moth prefer dates at an advanced maturity stage, such as the "Tamar" stage, to deposit their eggs, and then emerged larvae feed and develop inside the fruit and thus cause substantial postharvest losses in quality and quantity. During harvest and shipment, eggs can be deposited on the fruit whereas larvae and pupae may reside inside the commodity.

For these reasons, infestation by carob moth causes important economic losses of dates each year, with an average infestation of 20% in date fruit (Deglet Nour) production in Tunisia (Dhouibi 1992), and as high as 40% in the United States (Keck 1998). During the date fruit harvest period, infestation percentages can reach 80% (Munier 1973).

Methyl bromide (MB) remains the primary fumigant used to kill carob moth in date fruit (Dhouibi et al. 1991). However, MB has been associated with depletion of the Earth's ozone layer and is under restrictions of the Montreal Protocol (United Nations Environment Program [UNEP] 17998, Montreal Protocol) and expected to be phased out by 2015 in developing countries. Alternative fumigants, such as phosphine and sulfuryl fluoride, require long fumigation times of up to 3 and 1 d, respectively (Kader and Hussein 2009). Hence, an alternative to MB that can kill carob moth is

[1] Laboratoire d'économie Sciences et Technologies Alimentiares LR03AGR01.
[2] Department of Food Technology, National Agronomic Institute of Tunisia, University of Carthage, Tunis, Tunisia.
[3] Corresponding author, e-mail: haithem_bessi@hotmail.com.
[4] Department of Plant Protection and Post-harvest Diseases, Laboratory of Entomology-Acarology, National Agronomic Institute of Tunisia, University of Carthage, Tunis, Tunisia.
[5] Department of Plant Sciences, University of California, Davis, CA 95616.

needed by the date fruit industry. The alternative should be environmentally friendly, economically practical, and should also preserve product quality during storage.

Among fumigants, ethyl formate (EF) or a CO_2–EF mixture (BOC gases, 1997) have been re-evaluated in Australia by CSIRO Entomology as a fumigant alternative to control insects during on-farm storage (Annis 2002, Desmarchelier et al. 1998) and in the United States as a preshipment phytosanitary treatment (Simpson et al. 2004, 2007).

EF is a fumigant that had been used for disinfestations of pests from stored dried fruits since 1927 (Simmons and Gertler 1945), for grain protection (Muthu et al. 1984), and for stored split faba bean and sorghum (Ren and Mahon 2006). Vincent and Lindgren (1972) found that 6-h exposure to 26.5 and 23.2 g/m^3 EF at 26.7°C achieved 95% mortality for the larval stage of Indian meal moth (*Plodia interpunctella* (Hbn.)) and raisin moth (*Cadra figulilella* (Greg.)), respectively. Simpson et al. (2007) found that sufficient control of the fifth-instar omnivorous leafroller (*Platynota sultana* (Bonelli)) and the second-instar larvae of western flower thrips (*Frankliniella occidentalis* (pergande)) required exposure to 72.7 g/m^3 EF for 2 h. Aharoni et al. (1987) observed that a 3-h fumigation of grapefruit infested with the California red scale (*Aonidiella aurantii* (Maskell)) at 45.5 g/m^{3}of EF resulted in 100% mortality of all life stages of this insect. Earlier, Aharoni et al. (1980) demonstrated that a concentration of 15 g/m^3 EF during 1 h at a pressure of 30 mm Hg gave 100% mortality of western flower thrips (*F. occidentalis*). Hence, Mahon and Ren (2003) found that an effective concentration of 16–28 g/m^3 needed to be maintained for 48 h to efficiently control a mixture of insects (*Callosobruchus phaseoli*, *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus* spp, and *Cryptolestes* spp.) that attack different seeds of barley, wheat, and sorghum. More recently, EF has been tested for fresh fruit, such as grapes and strawberry, with infestations located close to the fruit surface (Simpson et al. 2004, 2007).

However, the efficacy of EF in controlling carob moth populations in stored dates is unknown, as well as the ability of the fumigant to penetrate inside the fruit in order to control the pest.

The purposes of this study were—1) to investigate EF sorption behavior in date fruit during fumigation at different concentrations and with dates of different moisture contents, 2) to predict adsorption of EF for semidry dates, using a model from the literature, 3) to test the efficacy of EF in controlling carob moth present in dates, and 4) to determine the most resistant life stage of *E. ceratoniae* to EF fumigation.

Materials and Methods

Raw Material. Date fruits (Deglet Nour) in the "Tamar" maturity stage were harvested from date palm trees located in Tozeur (Southwest Tunisia) in fall of 2009. The dates were shipped to California and transported to the UC Davis Postharvest Pilot Plant. The dates were stored at 5°C to maintain quality and to obtain uniform and undamaged fruit for use in future experiments.

When harvested, dates are a mixture of fruits with different water content. In this study, semidry and dry dates were tested individually for sorption of EF, and mixed moisture content dates were used for controlled fumigation tests with infested dates, as they represent the most common water contents of harvested dates (Deglet Nour). We needed to check if date conditioners may fumigate their dates without taking into account differences between the water content of individual dates. Hence, the commodity was divided into three groups of equal weight:

- Group 1: 410 g of dry dates (16% moisture content) ≈ 60 fruits.
- Group 2: 410 g of semidry dates (20% moisture content) ≈ 40 fruits.
- Group 3: 410 g of semidry (205 g ≈ 20 fruits) and dry (205 g ≈ 30 fruits) dates.

Fruit moisture content was determined by drying 5 g samples in a convection oven for 24 h at 105°C and expressed on a dry-weight basis (AOAC 1996).

EF Treatments. Each group of dates was placed in a 1-liter glass jar and sealed with a rubber stopper fitted with inlet and outlet tubing. Liquid EF (Sigma Aldrich, Saint Louis, MO) was injected (three replications per concentration) through the rubber stopper onto filter paper to the underside of the stopper (Simpson 2004, Vincent and Lindgren 1972). EF concentration was measured using a gas chromatograph (GC-9AM, Shimadzu Scientific Instruments, Columbia, MD) fitted with a 60/80 carbopack column with 5% carbowax (Supelco, Bellefonte, PA), a flame ionization detector, injection port temperature of 250°C (N_2 as carrier gas), and oven temperature of 85°C at 250°C. A separate outlet port was used to sample airspace EF concentration at the bottom of the jar through a 3-mm-diameter tube attached to the outlet. Airspace samples (1 ml) were obtained during tests using a glass, gastight, 1-ml syringe (Hamilton Co., Reno, NV)

Both dry (group 1) and semidry (group 2) dates were treated with different EF concentrations (28.6 ± 0.07, 57.3 ± 0.12, 85.9 ± 0.18, and 114.6 ± 0.05 g/m^3) for 120 min at 24°C to determine sorption of EF dates of different moisture content. EF concentration was measured in the airspaces at the bottom of the jar. Airspace samples were drawn from the treatment jars at 20-min intervals to monitor reductions in EF concentration over exposure time. An empty jar was injected with 114.6 g/m^3 EF for 120 min as a control. Each treatment was run in triplicate.

A mixture of dry and semidry dates was used for artificial infestation and then fumigated at 3 concentrations of EF (57.3, 85.9, and 114.6 g/m^3) for 120 min at 24 ± 1°C.

Mathematical Modeling. A mathematical model was applied in this study to correlate the quantity of EF adsorbed by dates with the initial concentration of fumigant injected and the exposure time.

Peleg's model is usually used to predict moisture loss or to study water vapor absorption by different matrices of food (Turhan 2002). Therefore, applying this model to describe the adsorption of a gas by a solid matrix of food is likely to be successful, even though no evidence that Peleg's model has been used to predict adsorption of a fumigant has been found in the literature.

Peleg's model (1988) proposes an equation (Eq. 1) to describe EF absorption kinetics, which includes two parameters, k_1 (Peleg rate constant) and k_2 (Peleg capacity constant). Both parameters were investigated for predicting EF sorption behavior during date fumigation.

$$X = X_0 + \frac{t}{k1 + k2t} \quad (1)$$

X: EF concentration absorbed by date fruit at time t, expressed as mg/g,

X_0: Initial EF concentration absorbed by date fruit, expressed as mg/g.

$\frac{1}{k_1}$: Reciprocal value of k_1 is the initial rate ($t = 0$) of absorption of EF, expressed as (min.g/mg of EF).

$\frac{1}{k_2}$: Reciprocal value of k_2 related to equilibrium EF concentration, used to determine the EF concentration absorbed by the dates at "equilibrium" when $t \rightarrow \infty$, expressed as (g/mg of EF).

Peleg's model was tested with experimental fumigations of semidry dates for 6 h. The quantity of EF adsorbed by the dates was deduced from the difference between the initial concentration injected into the jar and the quantity measured after a particular exposure time. The amount of absorbed EF was divided by the weight (410 g) of the sample (Fig. 1).

The fitting of the model to experimental data was done using Curve Expert, release 1.4 (Hyams Development, Hixson, USA). Model efficiency was evaluated by calculating the correlation coefficient (r) and standard error (SE).

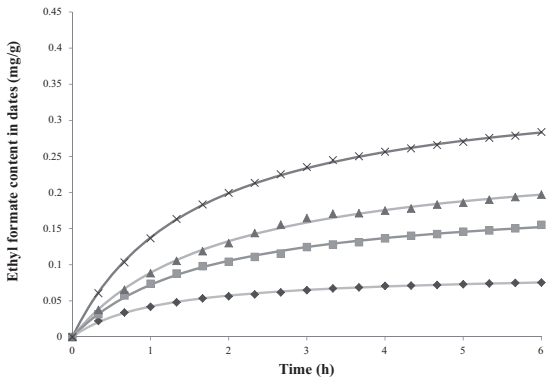

Fig. 1. Experimental and predicted EF adsorption for semidry dates.

Preparation of Life Stages for Artificial Infestation of Dates. Carob moth were reared in the Laboratory of Entomology of the National Agronomic Institute of Tunisia, under a temperature of 27 ± 1°C, a photoperiod of 16:8 (L:D) h, and 75 ± 5% relative humidity (RH), with an artificial diet composed of wheat bran, sucrose, glycerin, and water as major ingredients, in addition to a salt mixture, vitamin C, aureomycin, and methyl paraben (Fenny and Brinkham 1977; Abderahmane 2002).

One hundred sixty female moths were placed together with 40 males for 4 d in locally constructed oviposition cages (25 cm in diameter by 65 cm in height). The wall of the cylindrical oviposition cages consisted of removable paper sheets. Twenty-four hours later the 1-d-old eggs were collected by cutting pieces of paper from the sheet. The 1-d-old eggs were white in color, the 2-d-old eggs were pink, and the 3-d-old eggs were reddish.

For larvae, diet trays were stacked in transparent plastic cups (5 cm in height by 8 cm in diameter). When female moths had deposited sufficient eggs, the paper sheets of the oviposition cages were removed. These were then cut into equal-sized pieces which were deposited on the trays with the larval diet at a density of 1,500–2,000 viable eggs per one kilogram of diet. The larvae were reared under the above mentioned climatic conditions (Mediouni 2007). When an average emergence rate of 80% was reached, adult moths were held each in transparent plastic cups (5 cm in height by 8 cm in diameter).

Approximately 300 eggs were placed in three 59-ml plastic portion cups with a vented lid (Solo Cup Company, Urbana, IL) for each treatment, and there were three replications per treatment. Rate of emergence was determined by assessing survival of larvae hatched, after incubation for 15 d at ~27 ± 1°C, a photoperiod of 16:8 (L:D) h, and 75 ± 5% RH. The total number of eggs treated was determined using a stereomicroscope (Optica–S20L, Optical-Systems, France).

The third- and fifth-instars and pupae were placed individually inside each date fruit through the hole left by the perianth (in vitro infestation) using a thin brush. Fifty individuals per replication and treatment were used for each life stage tested, with three replications per treatment for a total of 150.

EF Fumigation. Based on the EF absorption behavior obtained with dates of different moisture content, group 3 dates (mixed moisture content), which are the most representative of harvested dates in commercial handling, were used for the insect disinfestation studies and fumigated at three concentrations of EF: 57.3, 85.9, and 114.6 g/m^3 for 120 min at 24°C. Larvae or pupae were placed inside the dates. Portion cups containing eggs were placed in the treatment vessel with 410 g of an even mixture of dry and semidry artificially infested dates for the fumigation treatment. Larvae mortality was checked daily and was determined by a lack of movement after 5 d of incubation at a temperature of ~27 ± 1°C, a photoperiod of 16:8 (L:D) h, and 75 ± 5% RH. Pupal mortality was determined by lack

of emergence as adults after incubation for 8 d under similar conditions.

The mean number of eggs was counted for the three EF concentrations of treated dates and for untreated samples. Eggs hatched from EF-treated samples after 15 d of incubation at 27°C and 75 ± 5% RH were divided by the mean number of eggs hatched in the control sample. Larval mortality (third- and fifth-instars separately) was determined daily during 5 d of incubation. The mean number of surviving larvae in the treated samples was divided by the number of surviving larvae in the untreated control, and the product was subtracted from 1 (Abbott 1925).

Pupal mortality was determined by a lack of emergence after 8 d of incubation under the same conditions for the samples of control and treated dates. The number of pupae emerging as adults in the treated samples was divided by the number of emerging adults observed in the control samples, and the product was subtracted from 1 (Abbott 1925)..

Data Analysis. Data were analyzed by a one-way analysis of variance (ANOVA) using SPSS version 11.0 (SPSS Inc., 2004). The means of insect mortality were separated using Tukey's LSD test.

Results and Discussions

EF Sorption Kinetics. After injection of EF at different concentrations into the treatment jars, the concentrations of EF were initially higher than expected, as a part of the jar volume was occupied by the sample (Figs. 2 and 3). After 120-min exposure of semidry dates to EF, the EF concentration inside the jars was reduced by 47, 45, 46, and 47% of the initial concentration for the treatments—28.6, 57.3, 85.9, and 114.6 g/m^3, respectively (Fig. 2). Hence, the decrease in headspace concentration was similar for the four treatment concentrations. A steady linear sorption behavior was observed for 120 min of exposure with a regression coefficient between 0.93 and 0.99 (Fig. 2). Similar results were observed for semidry dates (Fig. 3). The regression coefficients for semidry dates varied between 0.94 and 0.97.

Experimental results showed that dry and semidry dates had the same EF sorption behavior. For each concentration, EF sorption curves were very close throughout the exposure time for both dry and semidry dates. Hence, date moisture content had no effect on the date sorption capacity and, moreover, in commercial practice, dates with different moisture contents may be fumigated together when using EF. In addition, for all tested samples, the reduction in EF concentration varied between 42 and 51%. These decreases in concentration are greater than those found in the literature for other products. A reduction of about 30% of the initial EF concentration was observed for strawberry (Simpson et al. 2004) and 20% reduction was measured for table grapes (Simpson et al. 2007). In addition, Daby et al. (2009) found a 30% reduction after a 2-h exposure of wheat to EF.

Modeling. Experimental EF sorption curves have shown classical exponential sorption behavior. Thus, Peleg's model was successfully applied to the experimental data obtained for 6 h of fumigation of date fruit (Fig. 1). A good fit for the four EF concentrations tested was confirmed by the curves of experimental and Peleg model as adequate, and the correlation coefficients varied from 0.998 to 0.999 (Table 1).

Calculated model parameters show that the Peleg rate constant, k_1, decreased with increasing EF concentration (Table 1). As the reciprocal of k_1 ($1/k_1$) represents the initial mass transfer, higher EF fumigation concentrations allowed faster initial adsorption rates. In addition, higher injected EF concentrations resulted in a higher EF content in the fruit (Fig. 1). Calculated values of $1/k_2$ (Table 1), which represents the gas content inside the fruit at equilibrium, increased with higher EF concentration used in the fumigation.

Hence, Peleg's model may be very useful in predicting the quantity of EF absorbed and the behavior of sorption for date fruit, for any EF concentration used.

Lethal Effect of EF on Carob Moth. Overall, the eggs were the least resistant life stage to EF fumigation (Table 2). Baltaci (2008) demonstrated that the most resistant life stage of *Ephestia elutella* (Turner) to sulfuryl fluoride was the eggs, as compared with larvae

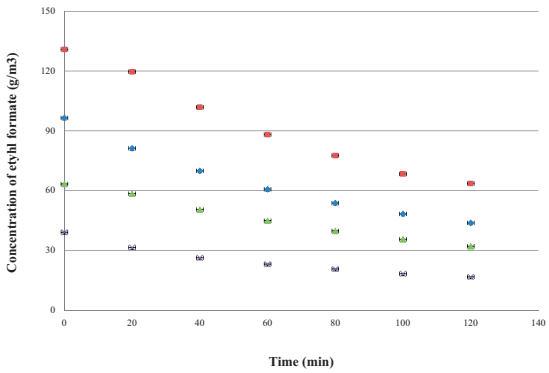

Fig. 2. Concentration of EF in the headspace during the fumigation period for semidry dates.

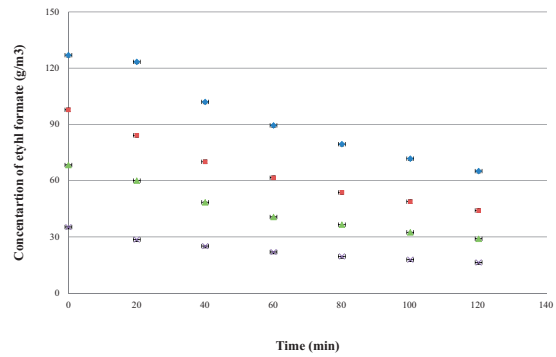

Fig. 3. Concentration of EF in the headspace during the fumigation period for dry dates.

Table 1. Peleg's model parameters for EF adsorption by date fruit at 24 ± 1°C

EF concentration (g/m^3)	k_1 (min (g/mg)$^{-1}$)	k_2 (g/mg)$^{-1}$	1/k_1 (min (g/mg))	1/k_2 (g/mg)	R^2
28.6	12.54	11.17	0.07	0.08	0.9996
57.3	8.72	5.12	0.11	0.19	0.9991
85.9	7.40	3.83	0.13	0.26	0.9989
114.6	4.47	2.78	0.22	0.35	0.9998

Table 2. Percent mortality of 3rd instar and 5th instar larvae, pupae and Percent of emergence of eggs of carob moth following date fruit exposure for 2 hours at 24 ± 1°C

EF (g/m^3)	Pupae mean ± SE[a]	Third-instar larvae mean ± SE[b]	Fifth-instar larvae mean ± SE[c]	Eggs mean ± SE[d]
0	4.60 ± 0.57[a]c	0.00 ± 0.0[a]c	0.00 ± 0.0d	82.0 ± 12.76[a]a
57.3	86.0 ± 6.04b	79.3 ± 3.05b	62.6 ± 9.01c	8.53 ± 9.98b
85.9	98.6 ± 1.21a	98.6 ± 2.3a	86.6 ± 1.15b	0.81 ± 1.40b
114.6	100.0 ± 0.0a	100.0 ± 0.0a	91.6 ± 1.32a	0.0 ± 0.00b

[a] Each value represents the mean of three replicates, each replicate contained 50 pupae.
[b] Each value represents the mean of three replicates, each replicate contained 50 third-instar larvae.
[c] Each value represents the mean of three replicates, each replicate contained 50 fifth-instar larvae.
[d] Each value represents the mean of three replicates, each replicate contained ~300 eggs.
Means in a column with the same letter were not significantly different at the 5% level.

and pupae. Liu et al. (2004) found that a vacuum of 50 mm Hg with doses of EF up to 10 g/m^3 for 4 h controlled eggs of *P. interpunctella*. In addition, Vincent and Lindgren (1972) showed that exposure to 9 g/m^3 of EF at 26.7°C for 6 h provided 95% mortality for eggs of two species belonging to the Pyralidae family (*P. interpunctella* and *C. figulilella*). In our study, fumigation treatments with 85.9 g/m^3 EF for 2 h were required to kill carob moth eggs.

The fifth-instars were the most resistant life stage to EF fumigation, compared with third-instars, eggs, and pupae (Table 2). There was no difference in mortality from fumigations at different levels of date moisture content, in agreement with the EF sorption behavior data. Fumigation with 85.9 g/m^3 EF for 2 h at 24°C resulted in 86.6 and 98.6% mortality of fifth-instars and pupae, respectively.

For Indian meal moth (*P. interpunctella*) and raisin moth (*C. figulilella*), Vincent and Lindgren (1972) found that the lethal concentration of EF was higher for the pupal stage than for larvae. Higher concentration was needed to reach sufficient control of fifth-instar omnivorous leafroller compared with other life stages (*Platynota sultana* (Bonelli); Simpson et al. 2007). Other researchers have demonstrated 100% mortality with EF treatments for a range of insects infesting various fruits, including *Aonidiella aurantii* (Maskell), *F. occidentalis*, *Callosobruchus phaseoli*, *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus* spp, and *Cryptolestes* spp. (Aharoni et al. 1987, 1980; Mahon and Ren 2003).

Finkelman (2010) demonstrated that exposure to 420 g/m^3 Vapormate (Linde Group, Munich, Germany) (16.7% w/w EF and 83.3% w/w CO_2) during 12 h at temperatures >24°C was needed to control nitidulid beetles (*Carpophilus hemipterus* L.) in date fruits, including eggs, larvae, and adults.

Our study demonstrates the ability of EF to penetrate inside the fruit and control insects, whereas for other studies, for example, when treating grains or grapes, the insect was on the outside surface of the fruit. Hence, EF treatment at a dose of 114.6 g/m^3 for 2 h at 24°C gave 100% mortality of all life stages of carob moth except the fifth-instar larvae, which reached 91.6% mortality (Table 2). A longer exposure to the fumigant or a higher concentration of EF may provide complete control of this life stage as well, and therefore reach the same carob moth lethality as a 12- to 24-h exposure to 30 g/m^3 MB at >16°C (Navarro 2006) or a 24-h exposure to 34 g/m^3 of sulfuryl fluoride at 20–25°C (Kader and Hussein 2009). Our results showed that EF exposure times required to control eggs of carob moth in date fruit is shorter than that of classic fumigants used in harvested dates. MB fumigation at 30 g/m^3 requires 12 to 24 h at >16°C, and phosphine, an approved and effective fumigant, requires 3–5 d at 20°C (Kader and Hussein 2009).

Further work is needed using the commercial formulation of EF, Vapormate, a nonflammable product that contains 16.7% by weight EF dissolved in liquid CO_2 (equivalent to 11% by volume EF in gaseous CO_2 when vaporized). The CO_2 can have a synergistic effect with the EF (Ryan et al. 2005, Simpson et al. 2007). When CO_2 enhances the efficacy of formulated active ingredient, this allows minimal use of active ingredients. The combination treatment may increase the efficacy of EF by allowing a shorter exposure time of fumigation, and providing complete control of carob moth life stages.

Further investigations will determine the effects of EF fumigation on the quality of date fruit immediately after fumigation and after cold storage. EF residues, aromatic compounds, and date color will be investigated to fully comprehend EF effects on date fruit.

Conclusion

Peleg's model showed a good fit with all experimental EF adsorption data ($0.9991 < R^2 < 0.9998$) and may thus

be used to predict adsorption kinetics under various conditions and EF exposure times. Because both dry and semidry dates showed the same EF adsorption behavior, date conditioners may fumigate both types of dates in the same fumigation chamber. Hence, EF fumigation presents an advantage when compared with microwave or radiofrequency treatments, where differences in date water content have a significant effect on the treatment. However, according to the study of Zouba et al. (2009), microwaves energy is preferentially absorbed by soft dates, which is expected, as their water content is higher. Dates conditioners use batches of dates where soft and dry dates are mixed, heat penetration is not the same, and disinfestations effects are different.

Fifth-instars were found to be the most resistant stage to EF fumigation, and while other life stages were completely controlled by EF, fifth-instars were not completely controlled, although mortality was high. Further investigations will include testing the Vapormate formulation of EF with CO_2 or a combination of low pressure (vacuum) with EF to reduce the EF concentration and exposure time in commercial-scale fumigations while achieving complete control.

Acknowlegments

Thanks to Mr. Bill Biasi for technical assistance in the Postharvest Pilot Plant of the Department of Plant Sciences, University of California, Davis, USA. Thanks also to Boujbel Company SAVACPA for providing date fruits (Deglet Nour) from Tunisia.

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Received 26 September 2013; accepted 23 January 2015.