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THE EFFICACY OF METHYLISOTHIOCYANATE AGAINST THE CODLING MOTH

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ABSTRACT

All stages of *Cydia pomonella*, the Codling moth on apples, can be eradicated by a methyl bromide (MB) fumigation followed by a period in cold storage at 0.5 or 2°C, necessary to control the egg stages which are insensitive to MB. Depending on the chosen standard of treatment, the cold storage time varies from 40 to 60 d. This work demonstrates that fumigation with methylisothiocyanate (MITC) at a Ct product of 2 g h m^{-3} is sufficient to effectively control all three egg stages of the Codling moth. This treatment overcomes the problem of immobilising the fruit, which can be exported a short time after harvest. Quality analysis shows that an MITC fumigation at a Ct product of 2 g h m^{-3} does not alter apple quality. The desorption tests, with a detection limit of 2 mg kg^{-1}, showed that no MITC was detected 1 h after the treatment.

INTRODUCTION

The biological efficacy of methylisothiocyanate (MITC) at an application dose of 20 to 40 g m^{-3} for 24 h has been demonstrated for all stages of *Sitophilus granarius* (Ducom, 1994). Numerous tests on other species of stored-product pests has confirmed the efficacy of the fumigant. The cited application doses are generally higher than the lethal concentrations required, but this is because the high sorption of MITC handicaps its diffusion and availability.

This study on the fumigation of apples with MITC was undertaken in order to satisfy the quarantine requirements against the Codling moth, *Cydia pomonella*, laid down by certain countries. Currently, this is done with methyl bromide (MB) fumigations, either at atmospheric pressure or in a partial vacuum, followed by a period of up to 50 d cold storage to eliminate the egg stages. The objective of our study was to control these stages using MITC. Because the moth oviposits on the fruit skin, a very low dose of the fumigant was applied for a very short time, thereby avoiding a high level of sorption inside the apples.

MATERIALS AND METHODS

Analysis of the biological efficacy of MITC was carried out on three embryonic stages of the Codling moth egg: W1 (1st instar, white stage), W3 (3rd instar, red ring stage) and W5 (5th instar, blackhead stage). The most resistant stage was defined.

All insects used in this study were laboratory-reared with an annual introduction of about 2% of wild insects collected in the Avignon region in the southeast of France. Breeding techniques were based on Sender (1969, 1970); Guennelon (1976); and Guennelon *et al.* (1981). No sanitary disorder (disease or virus) affected the insects during the experiments.

Three 15-m^3 air conditioned rooms were used. One was for emergence of the adults required for apple infestation; it was held at 25 ± 1°C, 70 ± 10% r.h. and a photoperiod of 16 h day/8 h night). The second was for infestation of fruit at 25°C, 70% r.h. and the same photoperiod. The third room was for attaining the target stage and apple incubation after a cold exposure period (27°C, 70% r.h.).

The Golden Delicious variety was chosen for this test.

Batches of ten apples per stage and per dose were used, and experiments were replicated twice.

The cages (84 × 27 × 29 cm) used for infesting the apples were disinfested, before each new infestation, by fumigation with MB at 40 g m^{-3} for 24 h followed by a desorption period. The fruit, stored at 0.5°C, was taken out of the refrigerator 24 h before the infestation and kept at 25°C and 70% r.h.

The infestation was carried out at 25 ± 1°C, 70 ± 10% r.h. and a photoperiod of 16 h day/8 h night. Fifty apples were placed in each infesting cage. 100–150 unsexed adult 2–5-d-old Codling moths were introduced into each cage for a 24-h oviposition period in order to obtain approximately 20–25 eggs per apple. After 24 h, the insects were removed by aspiration.

The three embryonic egg stages were prepared as follows: 1-d eggs (W1, white stage) were kept at 27°C for 12 h after the end of the infestation and then fumigated, aged 12–36 h; 3-d eggs (W3, red ring stage) were kept at 27°C for 60 h after the end of the infestation and then fumigated, aged 60–84 h; and 5-d eggs (W5, blackhead stage) were kept at 27°C for 108 h after the end of the infestation and then fumigated, aged 108–132 h.

MITC was evaporated from a crystal block which could be cut up to obtain the required quantities of the compound.

The 43-L gastight fumigation chambers were made of "altuglass." The desired dose of MITC was placed in the chamber after introduction of the apples. The ten apples used per dose gave a loading ratio of 4%. The chambers were equipped with an exterior pump, run continuously during the fumigation, which provided a flow rate of 14 L min^{-1}. The fumigation chamber was maintained at 20°C for 24 h preceding and for the duration of the treatment, and control apples were held at the same temperature. Dosages were calculated at 0.2, 0.4, 0.6, 0.8, 1 and 2 g m^{-3}. The exposure period was 2 h.

Measurements of MITC concentrations were made with a VARIAN 3 300 gas chromatograph, and the recordings were integrated using the integration card Star Varian and

Star Varian Version 4 software. Readings were taken at 0.5, 1 and 2 h after introduction of the gas.

After the MITC fumigation, the fumigated apples and controls were incubated in the incubating room for 9 d.

Mortalities of eggs on treated and control apples were determined after 9-d incubation by examining each isolated egg under a microscope. Eggs that had hatched were recorded as live, unhatched eggs as dead.

The results were subjected to probit analysis (Finney, 1971) using SAS software (1987) and expressed as lethal dosages at the following levels: LD_{50}, LD_{90}, LD_{95} and LD_{99}.

Physical, chemical and organoleptic quality of the apples

The aim of this study was to determine the physical, chemical, visual and olfactory characteristics of Golden Delicious apples treated with MITC for 2 h at 20°C and 2 h at 10°C, followed by storage for 20 d at 20°C. To evaluate apple quality in relation to fumigant dosage, the following four doses were tested: 0.5, 1, 1.5 and 2 g m^{-3}.

Each analysis was carried out on ten weighed apples using the following equipment as specified by the French standard NF V 20-201 (AFNOR, 1981): a centrifuge, a penetrometer FACCHINI, a refractometer EUROMEX (model 0–32 Brix), a pH meter (646 KNICK) with a pH INGOLD electrode, model 0–14.

Firmness

Firmness, expressed in kg cm^{-2}, was measured using a penetrometer in two places, on the sides with the most and least colour.

Apple juice

Each apple of each batch was analysed. Two pieces, representing a fifth of the apple, were cut between the holes made by the penetrometer. The 20 pieces so obtained were centrifuged and the juice filtered through sterilised hydrophilous cotton.

Dry soluble matter (sugar content)

A few drops of juice were placed in the refractometer. The reading obtained indicated the soluble dry material, expressed in degrees Brix.

Total acidity

Twenty ml of the same juice were homogenised by a magnetic stirrer and neutralised with a sodium hydroxide solution (NaOH, 0.1 N). Once the pH reached 8.2, no further solution was added. The value obtained, expressed in ml of sodium hydroxide, indicates the total acidity level of the solution.

Method of visual analysis

Visual analysis was conducted simultaneously with the physical and chemical analyses. The interior and exterior aspects of the apples were recorded, with the overall colour,

the colour homogeneity, the different marks, and coloration and condition of the seeds all being noted.

Method of olfactory analysis

Olfactory analysis was conducted after the chemical analysis. Two apple quarters were divided and the flesh sniffed by a panel which noted the strength and characteristics of the different smells (fruity, phenolic, vinegary, terpenolic, musty, lactonic, mouldy, aminated, etc.).

RESULTS AND DISCUSSION

Biological efficacy of MITC on *Cydia pomonella* eggs

Mortality data clearly reveal that Codling moth eggs have a high sensitivity to MITC. The least sensitive stage seems to be W5. Statistical analysis shows that the LD_{50} and LD_{90} of the W5 stage are greater than those of the W1 and W3 stages (Tables 1 and 2). In fact, a Ct product of 0.39 g h m^{-3} is necessary to obtain 50% mortality of the W5 stage compared with a Ct product of 0.43 g h m^{-3} and 0.26 g h m^{-3} for the W1 and W3 stages, respectively. A similar tendency can be found at the LD_{90} level.

However, the slope of the probit line for the W3 stage (1.21) is less than those of the probit lines for the W1 stage (2.65) and W5 stage (1.54). Thus, the LD_{99} of the W3 stage is greater than that of the W1 stage and could be compared to the W5 stages (Fig. 1). A

TABLE 1

Calculated LD_{50}, LD_{90}, LD_{95} and LD_{99} values, in Ct products (g h m^{-3}) for *Cydia pomonella* eggs on mature apples, fumigated with MITC for 2 h at 20°C

Stage	Slope (ln)	LD_{50}	LD_{90}	LD_{95}	LD_{99}
W1	2.65 (±0.64)	0.43 (0.31–0.49)	0.69 (0.59–1.07)	0.80 (0.65–1.44)	1.03 (0.78–2.55)
W3	1.21 (±0.14)	0.26 (0.19–0.32)	0.76 (0.64–0.96)	1.03 (0.83–1.41)	1.81 (1.34–2.95)
W5	1.54 (±0.50)	0.39 (0.09–0.51)	0.90 (0.73–2.16)	1.14 (0.87–4.87)	1.78 (1.15–23.5)

TABLE 2

Comparison between the sensitivity of *Cydia pomonella* eggs treated with MITC and MB at 20°C

	Ct product (g h m^{-3})		
Treatment	W1	W3	W5
LD_{50}, MB atmospheric pressure fumigation	23.02	16.04	15.16
LD_{50}, MB vacuum fumigation	23.90	14.80	11.40
LD_{50}, MITC	0.43	0.26	0.39
LD_{95}, MB vacuum fumigation	100.95	32.45	23.20
LD_{95}, MITC	0.80	1.03	1.14

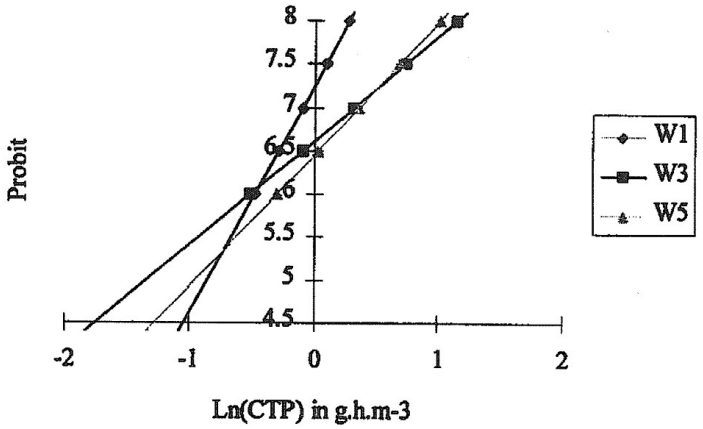

Fig. 1. Probit lines in relation to Ct product (CTP) of *Cydia pomonella* egg stages (W1, W3 and W5) exposed to MITC.

Ct product of 1.81 g h m^{-3} is necessary to obtain 99% mortality of the W3 population, as opposed to a Ct product of 1.78 g h m^{-3} to control 99% of the W5, and a Ct product of 1.03 g h m^{-3} to control 99% of the W1 population. The probit analysis of these studies therefore shows that the most resistant stage to MITC is W3.

Sensitivity comparison between Codling moth eggs treated with MITC and MB

Comparing the above results with those of Cugier (unpublished results), who used vacuum fumigation with MB, and those of Gaunce *et al.* (1980), who treated apples with MB at atmospheric pressure, reveals that, at the LD_{50} level, the MITC is 50 to 70 times more active than MB at atmospheric pressure or in partial vacuum. Comparison of vacuum fumigation with MB and fumigation with MITC shows that, at the LD_{50} level, MITC is 23 to 140 times more active than MB.

Effect of treatment on apple quality

No notable, significant difference between the control and the treated batches could be observed (Table 3). The firmness and the acidity of the apples were the same. A slightly higher refractometric index, indicating a slightly higher sugar content, was noted in the treated batches. The tasting panel did not note this difference as the fruit overall is not very sweet.

Visual analysis did not reveal any difference between the treated and untreated batches. The olfactory panel noted that the aroma of the treated apples was less marked and less enduring than that of the control.

Desorption of MITC from the apples

During a 2-h fumigation at 20°C, at an applied dose of 2 g m^{-3} and a loading of 5%, the fruit absorbed 3 ± 1% of the quantity of gas introduced into the chamber; the rest of the

TABLE 3
Quality parameters for apples treated with MITC (Ct 2 g h m^{-3}) and untreated apple batches

	Control		Treatment	
Sample	Batch 1	Batch 2	Batch 1	Batch 2
Average weight (g)	116.6	121.2	113.8	138
Refractometric index (° brix)	12.2	11.2	13.2	12.5
Free acidity (pH)	3.9	4.04	4.03	4.07
Total acidity (ml NaOH)	3.24	2.86	2.84	3.28
Firmness				
Average	3.43	3.23	3.58	3.58
SD	1.00	0.53	1.24	0.89
Minimum	2.4	2.5	2.1	2.4
Maximum	6.3	4.5	6	6.4

gas was absorbed by the chamber walls. Thus, depending on the weight of the treated apples, between 1.5 and 2.5 mg kg^{-1} of MITC remained at the end of the fumigation. After 4-h immersion in 250 ml of methanol (the method validated in the laboratory), no trace of MITC was found on the apples that had desorbed for 1 h after treatment.

CONCLUSION

In this study, 100% mortality was obtained on the three egg stages of the Codling moth with an MITC fumigation for 2 h at 20°C at an introduced dose of 2 g m^{-3}. The least sensitive stage was the the 3-d-old egg, with a LD_{99} of 1.81 g h m^{-3}. MITC fumigation of Golden Delicious apples at doses efficient against the Codling moth does not alter the quality of the fruit, and no trace of MITC was detected after 1 h of desorption at 20°C (desorption limit: 2 ppm).

MITC fumigation to control the egg stages of this pest obviated the need for about 50 d of cold treatment.

Further work is required to investigate the efficacy of this fumigant on the most MB-resistant larval stage, the 5th stage diapausing larva, before a standard method of control using a combination MB and MITC, or an MITC fumigation alone, can be envisaged.

The efficacy/quality relationship needs to be established for such standards.

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