Verification of Cold Treatment for Postharvest Control of *Ceratitis capitata* (Wiedemann), Mediterranean Fruit Fly, in Three Cultivars of Table Grapes

T.B. Platt[1], S.A. Johnson[2*] & D.H. Moelich[3]

(1) Experico Agri-Research Solutions, Lelie Street, Stellenbosch, South Africa. Current affiliation: Corteva Agriscience, 1 New Street, Paarl, South Africa, thomas.platt@corteva.com
(2) Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa, sjohnson@sun.ac.za
(3) South African Table Grape Industry, 63 Main Street, Paarl, South Africa, dawie@satgi.co.za

Accepted for publication: October 2024

Key words: *Ceratitis capitata*, cold treatment, table grapes

Cold treatment schedules for the control of phytosanitary insect pests such as fruit flies (Tephritidae) are standard practice in the international trade of fresh agricultural products. This study presents data on the efficacy of cold treatment of three table grape cultivars artificially infested with *Ceratitis capitata* (Mediterranean fruit fly) larvae, and demonstrates that, irrespective of cultivar, cold treatment at 1°C or below for 16 days, will effectively mitigate the risk of *C. capitata* in table grapes.

INTRODUCTION

Postharvest disinfestation treatments, such as fumigation, irradiation or temperature treatments, are used to mitigate the spread of phytosanitary insect pests during global trade of fresh fruit products. Fresh agricultural commodities require low temperature storage during long distance transport, thus sea-freight provides the opportunity to implement cold treatment protocols for risk mitigation of specific pests of concern while in transit. Fruit fly (Tephritidae) species are major economic pests of quarantine concern during international trade and are controlled using cold treatment protocols during shipping. The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is a key phytosanitary pest of South African table grapes for certain international markets, and several cold treatment options are available for postharvest control.

Longstanding approved cold treatment schedules against *C. capitata* for various crops imported into the USA are listed under T107 in the USDA Treatment Manual (USDA-APHIS, 2023). T107-a provides three treatment schedules that can be applied to a variety of crops, including grapes. These are, 1.1°C or below for 14 days; 1.67°C or below for 16 days; and 2.2°C or below for 18 days. In 2021, the International Plant Protection Convention (IPPC) adopted and published three treatment schedules for cold treatment for *C. capitata* on grapes, as part of ISPM 28 (IPPC, 2021). These are 1°C or below for 16 days; 2°C or below for 18 days; and 3°C or below for 20 days. These are also the stipulated mandatory cold disinfestation schedules used against quarantine fruit fly species for table grapes exported from Australia to Japan (DAFF Australia, 2024). Several international markets import a variety of table grape cultivars from South Africa. One table grape cultivar, Barlinka, is currently permitted for export from South Africa to Japan, subject to mandatory cold treatment at 0.8°C for 16 days. This cold treatment regime was based on results from a research report by Ware (2006) provided to Japanese National Plant Protection Organization, 18 years ago. However, the Barlinka cultivar has lost consumer preference to modern cultivars and is therefore no longer planted in South Africa.

In this report, the efficacy of cold disinfestation treatment against *C. capitata* is verified in three table grape cultivars: Crimson Seedless at 1.2°C for 14.88 days or less, and Sugrathirtyfour (ADORA SEEDLESS®) and Sugrathirtyfive (AUTUMN CRISP®) at 1.6 to 1.7°C for 14.88 days or less.

MATERIALS AND METHODS

Fruit

Crimson Seedless, a red coloured cultivar, Sugrathirtyfour, a black coloured cultivar and Sugrathirtyfive, a greenish-white cultivar, were selected for this study from the 20 most widely planted cultivars in South Africa, in 2024 (SATI Statistics, 2024). The table grapes, grown according to standard agricultural guidelines and production practices followed for export quality fruit, and packaged for fresh consumption, were purchased from commercial sources. Detached grape berries were dipped in 0.05 mL/L water Sporekill® solution

*Corresponding author: sjohnson@sun.ac.za
Acknowledgements: The work on Crimson Seedless was funded by the South African Table Grape Industry (SATI); the work on Sugrathirtyfour (ADORA SEEDLESS®) and Sugrathirtyfive (AUTUMN CRISP®) was funded by Sun World International, LLC.

(active ingredient didecyldimethylammonium chloride) (Hygrotech Sustainable Solutions, Pretoria, South Africa) for 4 minutes, followed by a dip into a 0.25 g/L chlorine granule solution (HTH Granular Mineralsoft+ at 600g/kg calcium hypochlorite) for 4 minutes, then transferred to a laminar flow bench to drip-dry. Once dry, a hole was drilled into each berry at the stylar end to a depth of one third its length using a sterilised 5 mm drill bit. The berries were then placed, open-side down, onto a moisture-absorbent material (MAM) sheet to drain excess moisture from inside the berries.

Insect inoculation of berries

Ceratitis capitata eggs were obtained from a sterile insect technique mass-rearing facility maintained by Fruit Fly Africa in Stellenbosch, Western Cape province, South Africa. Eggs were contained in a sterilised water suspension in glass vials and made up to a concentration of 800 eggs/ml. A 25 µl aliquot of this solution (containing approximately 20 eggs) was pipetted into each grape, and the end sealed with a sterilised cotton bud tip. These were then placed open-side down into black plastic seedling trays and incubated at 25°C. The incubation duration which yielded the greatest number of third instar larvae (measuring up to 8.7 mm in length) (Steck & Ekesi, 2015), as well as the highest average number of third instar larvae per grape, was found to be eight days. The third instar is considered by some researchers to be the most cold tolerant larval stage, however, Manrakhan *et al.* (2022) states that the choice of *C. capitata* instar for conducting cold tolerance studies is not important.

Thermoprobe calibration

Before each cold treatment, Type-T thermocouple wires (thermoprobes) with a stated accuracy of 0.5°C and a temperature range of -40°C to 105°C [Temperature Controls Pty., Ltd., Randburg, South Africa], were connected to Squirrel data loggers [Grant SQ 2F16, Monitoring and Control Laboratories Pty. Ltd., Johannesburg, South Africa] to record temperatures at 10-minute intervals during treatment. Thermoprobes were calibrated using the freezing point method where the probes are immersed in melting ice and the temperature recorded when equilibrium was reached. The calibration factor for each probe/datalogger terminal combination was determined in ice slurry for the correction of temperature data, where applicable. After calibration, the individual thermoprobes remained attached to the same terminals of the data loggers throughout the experiments and were randomly inserted into individual grape berries in prepared trays, to record pulp temperature during cold treatment.

Cold disinfestation trial procedure

Grapes were prepared for the trials as described above in order to obtain at least 6000 third instars after eight days of incubation at 25°C. After the incubation period, grapes were placed into black plastic seedling trays mounted in plastic containers and covered with MAM sheets in preparation for cold treatment trials. Half the total prepared berries were used for the untreated control. These were incubated for a further 48h at 22°C to facilitate larval development and then removed from the incubation room and dissected to assess the condition of third instar larvae (alive/dead) and to determine mortality, which was confirmed by movement after prodding. The other half of the inoculated berries, comprising the treatment, were transferred to the cold room and placed into a cage (1.455m x 0.825m x 1.46m) insulated with polystyrene. At least nine thermoprobes were inserted into individual grape berries to record pulp temperature at 10-minute intervals. Berries were kept in cold storage for between 13.04 and 14.88 days, measured from the first time that half of the thermocouples registered being at the targeted pulp temperature (within a deviation of ± 0.2°C), to avoid over-exposure. After cold storage, the treated berries were removed and incubated at 22°C for 48h to acclimatise. Cold treated berries were dissected and the number of alive and dead third instar *C. capitata* assessed to determine mortality as described. The above-described procedure comprised one experiment. The target number of larvae was at least 3000 for control and another 3000 for the cold treatment in each experiment. Three or four experiments were performed until total numbers per treatment exceeded 10,500 for both treated and control berries across each of the three cultivars. The targeted pulp temperatures for cold treatments were 1.2 ± 0.2°C for Crimson Seedless, and 1.6 ± 0.2°C for Sugrathirtyfour and Sugrathirtyfive. Duration of treatments were derived from the first and last hourly intervals of temperature recordings.

RESULTS

Observed percentage mortalities in treated fruit were not corrected for control mortality since percentage mortalities in control fruit were less than 5% (Table 1). A total of 41,450 third instar *C. capitata*, artificially inoculated in Crimson Seedless grapes, were assessed over three experiments. Of these, 18,986 were treated with cold storage during which the mean grape berry pulp temperature was 1.33°C ± 0.01°C, for a mean duration of 13.99 ± 0.01 days at temperature (Table 2), while the remaining 22,464 comprised the untreated control, kept at room temperature of ± 22°C. Mortality was 100% across all experiments for *C. capitata* larvae subjected to cold storage, while the mortality among the untreated controls was 1.87% ± 0.02 (Table 1).

A total of 29,434 third instar *C. capitata*, artificially inoculated in Sugrathirtyfour grapes, were assessed over four experiments (Table 1). Of these, 15,076 were treated with cold storage during which the mean grape berry pulp temperature was 1.60°C ± 0.01°C, for a mean duration of 14.62 ± 0.10 days at temperature (Table 2), while the remaining 14,358 comprised the untreated control, kept at room temperature of ± 22°C. Mortality was 100% across all treatments for *C. capitata* larvae subjected to cold storage, while the mortality among the untreated controls was 1.12% ± 0.01 (Table 1).

A total of 28,267 third instar *C. capitata*, artificially inoculated in Sugrathirtyfive grapes, were assessed over four replicates (Table 1). Of these, 13,916 were treated with cold storage during which the mean grape berry pulp temperature was 1.69°C ± 0.01°C, with a mean duration of 13.21 ± 0.06 days at temperature (Table 2), while the remaining 14,351 comprised the untreated control, kept at room temperature

of ± 22°C. Mortality was 100% across all replicates for *C. capitata* larvae subjected to cold storage, while the mortality among the untreated control replicates was 0.68% ± 0.01 (Table 1).

DISCUSSION and CONCLUSIONS

The IPPC approved treatment schedules for disinfestation of *C. capitata* in *Vitis vinifera* L. is based on extensive research conducted in Australia (De Lima, 2007; De Lima *et al*., 2011; De Lima *et al*., 2017). Nine cultivar-specific trials (including

TABLE 1

Control and treated numbers of total, live, dead and observed percentage mortality and standard errors (SE) of third instar *Ceratitis capitata* cold treated in three artificially-infested table grape cultivars: Crimson Seedless, Sugrathirtyfour, Sugrathirtyfive

	Total number of larvae treated	**Number of live larvae**	**Number of dead larvae**	**% mortality ± SE**
Crimson seedless (*3 experiments)*				
Control	22 464	22 061	403	**1.87 ± 0.02**
Treatment	18 986	0	18 986	**100**
Total	*41 450*	*22 061*	*19 389*	
Sugrathirtyfour (*4 experiments)*				
Control	14 358	14 222	136	**1.12 ± 0.01**
Treatment	15 076	0	15 076	**100**
Total	*29 434*	*14 222*	*15 212*	
Sugrathirtyfive (*4 experiments)*				
Control	14 351	14 275	76	**0.68 ± 0.01**
Treatment	13 916	0	13 916	**100**
Total	*28 267*	*14 275*	*13 992*	

TABLE 2

Minimum, maximum and mean hourly temperatures and standard errors (SE) of each, as well as temperature at start of treatment per experiment, and the duration of each replicate in cold storage, as assessed in *Ceratitis capitata* artificially-infested Crimson Seedless, Sugrathirtyfour and Sugrathirtyfive grape berries. Duration of treatment was derived from the first and last hourly intervals.

Cultivar and experiment number	Hourly minimum temperature ± SE (°C)	Hourly maximum temperature ± SE (°C)	Hourly mean temperature ± SE (°C)	Temperature at start of treatment (°C)	Number of days at temperature
Crimson seedless					
1	1.29 ± 0.01	1.33 ± 0.01	1.31 ± 0.01	1.2	14.12
2	1.34 ± 0.01	1.44 ± 0.01	1.39 ± 0.01	1.2	14.13
3	1.25 ± 0.01	1.33 ± 0.01	1.29 ± 0.01	1.2	13.71
Mean	**1.29 ± 0.01**	**1.37 ± 0.01**	**1.33 ± 0.01**	**1.2**	**13.99 ± 0.01**
Sugrathirtyfour					
1	1.52 ± 0.01	1.62 ± 0.02	1.56 ± 0.01	1.4	14.46
2	1.73 ± 0.01	1.82 ± 0.01	1.78 ± 0.00	1.6	14.67
3	1.42 ± 0.01	1.49 ± 0.01	1.45 ± 0.01	1.6	14.46
4	1.55 ± 0.01	1.63 ± 0.01	1.59 ± 0.01	1.6	14.88
Mean	**1.56 ± 0.01**	**1.64 ± 0.01**	**1.60 ± 0.01**	**1.55**	**14.62 ± 0.10**
Sugrathirtyfive					
1	1.60 ± 0.01	1.66 ± 0.01	1.63 ± 0.00	1.5	13.04
2	1.75 ± 0.01	1.82 ± 0.01	1.79 ± 0.01	1.6	13.25
3	1.72 ± 0.01	1.80 ± 0.01	1.76 ± 0.01	1.8	13.29
4	1.53 ± 0.01	1.61 ± 0.01	1.57 ± 0.01	1.8	13.25
Mean	**1.65 ± 0.01**	**1.72 ± 0.005**	**1.69 ± 0.01**	**1.68**	**13.21 ± 0.06**

 DOI: https://doi.org/10.21548/45-2-6722

Crimson seedless) were presented and reviewed by De Lima *et al.* (2017). The authors claim that given the comprehensive body of work and relevant data available for this pest-host combination, the three treatment schedules shown to be efficacious against *C. capitata*, should apply to all *V. vinifera* cultivars presented for export. The IPPC recognised this and supported this claim, since these schedules were adopted as an international phytosanitary standard in ISPM 28 (IPPC, 2021). Results from the verification study presented here on disinfestation of *C. capitata* in three table grape cultivars grown in South Africa, during which all cold treatments are ≥1.29°C and ≤ 1.79°C and last between 13.04 and 14.88 days, fall within the range of schedules that this standard prescribes. The approved IPPC treatment of 1°C or less for 16 days (IPPC, 2021) should effectively mitigate the risk of *C. capitata* in table grapes with a good margin of safety.

LITERATURE CITED

DAFF [Department of Agriculture, Fisheries and Forestry] Australia. 2024. Manual for importing country requirements. https://micor.agriculture.gov.au/Plants/Pages/Japan_JP/Grapes.aspx

De Lima, C.P.F., Jessup, A.J., Mansfield, E.R. & Daniels, D. 2011. Cold treatment of table grapes infested with Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) Diptera: Tephritidae. N. Z. J. Crop. Hortic. Sci. 39(2), 95-105. https://doi.org/10.1080/01140671.2010.526620

De Lima, C.P.F., Mansfield, E.R. & Poogoda, S.R. 2017. International market access for Australian tablegrapes through cold treatment of fruit flies with a review of methods, models and data for fresh fruit disinfestation. Aust. J. Grape. Wine. Res. 23, 306-317. https://doi: 10.1111/ajgw.12284

IPPC [International Plant Protection Convention]. 2021. ISPM 28. Phytosanitary treatments for regulated pests. Annex 36. Cold treatment for *Ceratitis capitata* on *Vitis vinifera*. Rome, IPPC, FAO.

Manrakhan, A., Daneel, J-H., Stephen, P.R. & Hattingh, V. 2022. Cold tolerance of immature stages of *Ceratitis capitata* and *Bactrocera dorsalis* (Diptera: Tephritidae). J. Econ. Entomol. 115(2), 482-492. https://doi.org/10.1093/jee/toab26

SATI Statistics. 2024. Statistics of table grapes in South Africa. Compiled by J.F. Ferreira & C. Burger. https://user-hpa96tt.cld.bz/SATI-STATISTICS-OF-TABLE-GRAPES-IN-SOUTH-AFRICA-2024/

Steck, G. J. & Ekesi, S. 2015. Description of third instar larvae of *Ceratitis fasciventris*, *C. anonae*, *C. rosa* (FAR complex) and *C. capitata* (Diptera: Tephritidae). ZooKeys 540, 443-466. https://doi.org/10.3897/zookeys.540.10061

USDA-APHIS [United States Department of Agriculture - Animal and Plant Health Inspection Service]. 2023**.** Treatment manual 05/2023-66, Chapter 5, p. 5-2-90. T100-Schedules for fruit, nuts and vegetables. https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf).

Ware, A. 2006. Verification of cold treatment disinfestation treatment of Medfly-infested Barlinka table grapes destined for Japan. Unpublished report.