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Review

Postharvest Disinfestation Treatments for False Codling Moth and Fruit Flies in Citrus from South Africa

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Abstract: South Africa is the 13th largest producer and second largest exporter of citrus fruit globally. The false codling moth, *Thaumatotibia leucotreta*, and the fruit flies, *Ceratitis capitata*, *C. rosa* and *Bactrocera dorsalis*, can potentially infest citrus fruit and therefore pose a phytosanitary risk for export markets. Consequently, a wide range of postharvest phytosanitary treatments for disinfestation of citrus fruit from these pests have been investigated. These include cold treatments, irradiation, fumigation, heat treatments, and combinations of some of these. Due to the potential phytotoxic effects of all these treatments, the use of a systems approach that depends on two or more independent measures for acceptable phytosanitary risk mitigation is a preferable option. To date, the only postharvest disinfestation treatments used commercially for *T. leucotreta* and fruit flies for South African citrus, are stand-alone cold treatments and partial cold treatments, as a component in a multi-tiered systems approach. Research on development of novel and improvement of existing postharvest measures continues as a high priority. This includes postharvest detection technologies, in addition to treatment technologies.

Keywords: *Thaumatotibia leucotreta*; *Ceratitis capitata*; *Ceratitis rosa*; *Bactrocera dorsalis*; phytosanitary; disinfestation; cold treatment; irradiation; fumigation; systems approach

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1. Introduction

South Africa is the 13th largest producer and the second largest exporter of citrus fruit globally [1]. One of the big challenges in producing high quality fruit, fit for market, is the effective control of all pests and diseases. Bedford et al. [2] list at least 110 insect and mite pests of citrus in southern Africa. These could be separated into different categories, based on the type of threat they pose to the crop, such as cosmetic, production, disease vector. However, possibly the most important category for an export crop and certainly one that requires the highest focus in the pest management programme, is that of pests that constrain global trade and are identified as being of phytosanitary and hence quarantine importance by trading partners. A quarantine pest is defined as a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled [3]. These pests are not necessarily any more difficult to control than cosmetic or production pests. However, the zero tolerance for these pests exercised by trading partners (importing countries) mean that management of these pests based on economic or intervention thresholds is not sufficient and an even higher level of control is required to ensure complete freedom of the commodity from the pests. If the pestiferous life stages of the quarantine organism occur inside the plant part being exported, which for citrus is the fruit, this makes the task of ensuring pest freedom even more challenging.

The most important fruit infesting pests of citrus in southern Africa are false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) and fruit flies (Diptera: Tephritidae) [4]. Three species of fruit flies affect citrus in the region: the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), the Natal fruit fly, *Ceratitis rosa* Karsch and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) [4]. In South Africa, commercial export grade lemon, *Citrus limon* (L.) Burman f., was shown to be a non-host for *T. leucotreta* and the three fruit fly pests of citrus [5,6]. Lime, *Citrus aurantifolia* (Christm.) Swingle is not listed as a host for the four pests [7,8] and in South Africa no infestation of lime by *T. leucotreta* and fruit flies has ever been recorded. Although for citrus, fruit infestation by *T. leucotreta* and fruit flies can be effectively reduced or prevented in the field using an array of preharvest tools, many export markets still require a postharvest treatment in order to provide a specifically defined level of statistical certainty that the fruit will not be infested with any live individuals of these species, with the most extreme treatment being at Probit 9 level of efficacy [9].

Here we review the postharvest treatments developed for *T. leucotreta*, *C. capitata*, *C. rosa* and *B. dorsalis* in citrus. We put a stronger emphasis on the development of cold treatments for these pests for either stand-alone disinfestation or partial disinfestation as part of a multi-tiered systems approach. We then conclude with potential postharvest technologies that can be explored in the future for effective disinfestation or detection of both fruit flies and *T. leucotreta* in citrus.

2. Stand-Alone Cold Disinfestation

Cottier [10] was the first to report the efficacy of cold treatment for disinfestation of *T. leucotreta* and fruit fly in citrus fruit. *Citrus sinensis* (L.) Osbeck, orange, was either naturally or artificially infested with these pests. He reported shipping infested fruit from South Africa to New Zealand at a pulp temperature of −0.55 °C for a duration of 21 d. No live *T. leucotreta* and fruit fly were recorded, leading the author to conclude that the treatment was effective. However, Cottier [10] questioned the reliability of his conclusion, due to the small numbers of larvae treated (1 038 fruit fly larvae and 5 504 *T. leucotreta* larvae) and recommended further work. In his experiment, Cottier [10] did not distinguish between the two fruit fly pests: *C. capitata* and *C. rosa*. Cottier [10] also did not state the origin of the treatment being tested. It appears that it might have been as per the recommendations of the International Institute of Refrigeration [11], as referenced by Myburgh [12].

Cottier's [10] work was taken further by Myburgh [12,13], who tested the efficacy of −0.55, 1.11 and 4.44 °C fruit pulp temperatures for disinfestation of citrus fruit of *T. leucotreta*, eggs, larvae and pupae. After recording no survival of 272,000 eggs at various stages of development after only 8 d at 1.11 °C, he concluded that eggs were highly sensitive to cold and that no further trials were required against this life stage. Myburgh [12] and Myburgh and Bass [14] demonstrated that *T. leucotreta* pupae were more cold-tolerant than the larvae. However, there was no practical relevance to this finding, as *T. leucotreta* has never been recorded to pupate in citrus fruit, as fifth instars exit the fruit to pupate in the soil [4,15]. Myburgh [12] also demonstrated that fourth and fifth instars were the most cold-tolerant larval stages and therefore all further cold treatment studies with *T. leucotreta* could be conducted with these life stages. This finding was later confirmed by Moore et al. [16].

Another important finding on *T. leucotreta* in the study of Myburgh [12] was that cold treatment trials could be conducted with laboratory reared larvae on an artificial diet, without risk of overestimating the efficacy of the treatment. This finding was later confirmed by Moore et al. [16] and Moore et al. [17]. It is a practically important conclusion for two reasons. Firstly, sufficiently high levels of *T. leucotreta* infestation are no longer found in the field, as the pest is generally suppressed to a much lower level than was the case previously [18,19], due to its phytosanitary status for export markets and the extensive arsenal of effective control measures available [20,21]. Secondly, artificial infestation of large numbers of citrus fruit in the laboratory is not feasible, as too many fruit would decay before the trial could be evaluated [12,16,22]. Several *T. leucotreta* laboratory cultures are in existence throughout South Africa, which can be accessed for trials on artificial diets.

Through testing large numbers of *T. leucotreta* larvae, Myburgh [12,13] demonstrated that there were no survivors when approximately 122 000 larvae were exposed to −0.55 °C for 8 d followed by 17 d at 1.1 °C. In another two trials, he recorded no survival of approximately 43,000 *T. leucotreta* larvae after 20 consecutive days at −0.55 °C [12,13], validating a 99.993% mortality at a 95% confidence level, which is an assurance of a probit 8.7 level of efficacy [23–25]. Myburgh [13] reported that the cold treatments for *T. leucotreta* would adequately mitigate against the risk of two fruit fly pests of citrus: *C. capitata* and *C. rosa*. This conclusion was based on complete control of these two fruit fly species in naturally infested deciduous fruit in small scale trials after 9 d at −0.55 °C and 12 d at 1.11 °C [13]. The successful control of fruit flies in citrus, more specifically in *C. sinensis*, *Citrus paradisi* MacFad., *C. limon* and *Citrus reticulata* Blanco, by postharvest cold treatments at sub-zero temperatures (between −0.5 and −0.6 °C) for 12–14 d was confirmed thereafter in larger scale trials involving between 18,147 and 51,067 fruit fly larvae [26–29].

Most existing stand-alone cold treatment schedules for *T. leucotreta* in export citrus from South Africa have been based on Myburgh's [12,13] data, generally requiring 22 d at a sub-zero temperature.

There were, however, indications from Myburgh's [12] data that less stringent protocols (higher temperatures and/or shorter durations) may provide the requisite level of efficacy, even if demonstration of a Probit 9 level of efficacy was required. This possibility of less stringent cold treatment protocols was confirmed in subsequent trials by Hofmeyr and Hofmeyr [30] who recorded no survival of 10 442 fifth instar *T. leucotreta* in fruit, after exposure to −0.6 °C for 16 d. Consequently, Moore et al. [31] conducted a range of trials with warmer temperatures and shorter durations, demonstrating that the following regimes all succeeded in providing Probit 9 efficacy against fourth and fifth instar *T. leucotreta*: 16 d at −0.2 °C, 18 d at −0.4 °C, 20 d at −0.4 °C and 19 d at 1.0 °C. By using the mean hourly maximum probe reading in the experiments as the upper treatment threshold in the protocol, these were recommended for conversion into the following treatment schedules: 16 d at or below −0.1 °C, 18 d at or below −0.3 °C, 20 d at or below −0.3 °C and 19 d at or below 1.2 °C. These shorter duration and higher temperature treatments would be advantageous in overcoming some of the detrimental effects of cold treatments, such as chilling injury to fruit and protracted shipping duration to market [31].

Cold treatments at temperatures above 0 °C (0.9–3 °C) for 14–20 d have been found to be efficacious against both *C. capitata* and *B. dorsalis* in citrus [32–39]. For *C. capitata*, treatments at between 2 and 3 °C for 18–20 d were found to be effective at a Probit 9 level [39]. The latter treatments were adopted as internationally accepted cold treatments and are part of annexes of the International Standards for Phytosanitary Measures (ISPM) on Phytosanitary treatments of regulated pests (ISPM 28) [40–46]. The cold treatment of 19 d at or below 1.2 °C developed for *T. leucotreta*, as mentioned above, falls within the *C. capitata* cold treatment schedules and based on Myburgh's [13] study should also be effective against *C. rosa*. Moreover, cold treatments effective against *C. capitata* would also be efficacious against *B. dorsalis* [47,48].

Currently there are several export markets with whom South Africa has bilaterally agreed protocols for cold disinfestation treatment of citrus fruit for either fruit flies or *T. leucotreta* or both. These are generally at sub-zero temperatures for stipulated durations. This is despite the internationally accepted treatments for fruit flies that are at temperatures above 0 °C, and demonstration of the efficacy of 1.2 °C for 19 d against *T. leucotreta* [31].

3. Partial Cold Treatments

Partial cold treatments would be treatments that alone are not considered to provide adequate efficacy for use as a stand-alone disinfestation treatment. To date, these have been determined for *T. leucotreta*, but not yet for any of the fruit fly species. Some of these treatments were determined incidentally in establishing appropriate protocols for large scale validation as stand-alone disinfestation treatments and some of these were specifically developed as partial or incomplete cold treatments for use as one of the measures in a multi-tiered systems approach.

In the early work conducted by Myburgh [12] to develop a stand-alone cold disinfestation treatment for *T. leucotreta*, the efficacy of a series of partial cold treatments at three different temperatures, −0.55, 1.11 and 4.44 °C, was reported. This was done most comprehensively at 1.11 °C, where larval mortality varying between 96.30% and 99.96% was recorded following treatment at this temperature for 8–17 d, with pre-shipping cold storage varying between 1 and 12 d.

More recently, a series of partial cold treatments were developed during a study, spanning a 9-year period [17]. The treatments, consisting of a range of temperatures and durations, were selected to include regimes that offered a reduced risk of chilling injury [49] particularly for cold sensitive cultivars. Some time–temperature combinations were shown to induce 100% mortality (albeit usually not demonstrated at the Probit 9 level of efficacy). However, many of the treatments did not provide complete mortality, even though often being in excess of 99% effective. The mortality of the most cold-tolerant larval stages (fourth and fifth instars) of *T. leucotreta* was determined at a range of cold temperatures (0, 1, 2, 3, 4, 4.5 and 5 °C) in a series of durations from 16 (and 14 d in the case of 1, 2 and 4 °C) to 26 d. The range of durations (from 16 to 26 d) was selected, based on estimates of the duration required to achieve sufficiently high levels of mortality to be effectively used in a multi-tiered systems approach. These durations also matched the likely durations of shipping journeys to certain export markets. Temperatures of 0, 1, 2 and 3 °C for 16, 19, 20 and 24 d, respectively, induced 100% mortality of the test populations. Mortalities in excess of 90% were obtained with temperatures of 4, 4.5 and 5 °C, after exposure for longer durations. A significant difference in insecticidal efficacy was also demonstrated between 1, 2, 3 and 4 °C. There was no significant difference in insecticidal efficacy between 4 and 4.5 °C, but both of these temperatures were more efficacious than 5 °C [17].

There has also been a recent investigation on the efficacy of cold treatments at temperatures above 3 °C (3.5 °C) for at least 24 d for control of fruit flies in citrus [50]. Some preliminary results of this investigation indicated that *C. capitata* is more cold tolerant than *C. rosa* and *B. dorsalis* at 3.5 °C [50]. The studies also showed that a cold treatment at 3.5 °C for 24 d affects complete mortality of *C. capitata*, the most cold tolerant fruit fly pest of citrus in southern Africa, in larger scale trials [50]. These indicate that some partial cold treatments for *T. leucotreta* might be stand-alone cold treatments for fruit flies. However, for temperatures above 3.5 °C, durations higher than 24 d might be required for complete mortality of fruit flies as shown in the study by Back and Pemberton [51].

4. Irradiation

The use of ionizing radiation as a postharvest treatment for quarantine pests has been well studied for fruit flies [52] and was also explored for *T. leucotreta* in citrus fruit, as an alternative to cold disinfestation treatment [22]. Unlike other stand-alone disinfestation treatments, there is no expectation that irradiation will result in acute mortality. After fruit consignments are treated with irradiation, live, but non-viable larvae and/or pupae can still be encountered by inspectors but this does not imply a treatment failure [53,54]. While with fruit flies, the main objective with ionizing irradiation as a postharvest treatment is prevention of maturation (for example inability for eggs or larvae to develop to adulthood) [52], with *T. leucotreta*, the focus of investigation with this treatment has been on its sterilizing effect [22].

For both *T. leucotreta* and fruit flies, the later larval stages were found to be more tolerant to irradiation than the earlier life stages [22,55,56]. For fruit flies, differences in tolerance to irradiation were also found across species [56,57]. *Bactrocera dorsalis* was found to be more tolerant to irradiation than *C. capitata* [57]. There is no published information to date on the effect of ionizing radiation on *C. rosa*.

Fruit fly larvae in hypoxic conditions, for example in fruit with high water content or in fruit stored under low oxygen, were found to be more tolerant to irradiation than those in normoxic conditions [56–58]. However, the protection from irradiation that the fruit fly larvae derived from some low oxygen level environments was found to be nullified at high irradiation doses [55–59]. Studies by Hofmeyr et al. [22] clearly showed that fertility of moths originating from field collected larvae was reduced to a significantly greater extent than fertility of moths originating from laboratory reared larvae on an artificial diet, when both were treated with 40 Gy. Consequently, further trials were justifiably conducted with laboratory reared larvae on an artificial diet.

For *B. dorsalis* and *C. capitata*, many of the studies demonstrated that third instars exposed to gamma irradiation doses varying from 40 Gy to about 250 Gy did not develop into adults [55–57,60–62]. For *T. leucotreta*, adult moths were found to be sterilized when fifth instars were treated with 60 Gy, while 150 Gy completely halted further development of fifth instars [63]. A large-scale validation trial was subsequently carried out to demonstrate efficacy of a postharvest irradiation treatment on *T. leucotreta* at 100 Gy [64]. More than 124,000 fifth instars were treated with a target dose of 100 Gy ionizing radiation on an artificial diet. After treatment exposure, mean numbers of pupae and moths developing, were reduced by 49% and 86%, respectively, relative to untreated larvae. The ratio of males to females was also altered from approximately unity to 2.4:1. Moths were unable to fly and mating was reduced by 83%. Moths were also completely sterile, as no oviposition took place. Consequently, the results validated 100 Gy radiation as a potential postharvest disinfestation treatment for *T. leucotreta* at the Probit 9 efficacy level. This validated dose for *T. leucotreta* falls below the current generic dose of 150 Gy recommended for postharvest treatment of all fruit fly species [54,57]. The United States Department of Agriculture (USDA) approved a treatment of 400 Gy for fruit flies and other insect pests (except adults and pupae of Lepidoptera) in litchi, grape and persimmon from South Africa [53]. Citrus is not on this list, but if added, this dose will be unnecessarily excessive, given that the data clearly show that 150 Gy will provide effective control of both fruit flies and *T. leucotreta* in citrus. This validated citrus treatment could be a valuable addition to existing irradiation treatment options.

When irradiation is applied in fruit for disinfestation of internal pests, the required dose should reach fruit packed in the middle of a pallet, implying that irradiation emitted would need to be three to four times the dosage required [65], i.e., 300–400 Gy. As mentioned for cold treatments, irradiation also does not come without risk to fruit quality. This high rate would be particularly damaging to fruit packed on the outside of the pallet, as explained in more detail in the relevant section below.

5. Irradiation–Cold Combination

As a result of the detrimental effect of a stand-alone irradiation treatment on fruit quality, at the dosage required to achieve efficacy at the Probit 9 level, a combination irradiation–cold treatment was investigated for *T. leucotreta*, which would permit the use of a lower and safer irradiation dose [66]. Irradiation doses from 40 to 70 Gy were tested in combination with (i.e., followed by) cold treatments of 0.6–7.0 °C for periods of between 10 and 22 d. Consequently, not only lower and safer irradiation doses were tested, but higher temperatures and shorter durations of exposure, relative to the stand-alone cold disinfestation protocols for *T. leucotreta*, were also tested, thus also reducing the possibility of chilling injury. Ultimately, a combination treatment consisting of 60 Gy followed by 16 d at 2.5 °C was identified for larger scale testing. This was then done with more than 100,000 fifth instars on an artificial diet, leading to 99.7% larval mortality [67]. A further 50% of the survivors died after pupation. Sex ratio of the developing adults was eight males to every female, with fewer than 5% of the moths able to fly and fecundity was totally suppressed. Consequently, the efficacy of this treatment was validated at the Probit 9 level, making it an option for consideration as a commercial phytosanitary disinfestation treatment for *T. leucotreta* in citrus fruit. The proposed combined treatment of irradiation at 60 Gy followed by 16 d at 2.5 °C would also highly likely be effective for fruit fly pests of citrus in South Africa, although this has not been demonstrated at the Probit 9 level. However, this could be considered unnecessary, as simply reducing the temperature to 1.7 °C or reducing it to 2.2 °C and extending the exposure period to 18 d, would alone be compliant with widely accepted fruit fly treatments, even without irradiation [53]. Smaller scale trials have previously demonstrated that no adult emergence occurred when third instar *C. capitata* and a more radiotolerant fruit fly species, *Zeugodacus cucurbitae* (Coquillett), were treated with 30–35 Gy, followed by a treatment at 4 °C for 11 d in artificial larval diet and in papaya [68]. In small scale trials, Palou et al. [69] also recorded no adult emergence when *C. capitata* third instars in citrus were treated with X rays at 30 Gy, followed by a short cold treatment at 1 °C for 2 d. This combination treatment of low irradiation and short cold storage was found to have minimal impact on the quality of citrus, more specifically on *Citrus reticulata* Blanco [69]. For fruit fly pests of citrus in South Africa, a large-scale validation trial of the *T. leucotreta* low dose irradiation and cold storage protocol is certainly warranted. Such a protocol, if approved, may be better for more cold sensitive citrus cultivars to markets, such as the USA, that accept irradiation as a postharvest treatment.

6. Fumigation

Fumigation was the main method of disinfesting fruit commodities from fruit flies worldwide until the 1980s [70]. Methyl bromide and ethylene dibromide were the fumigants that were predominantly used for treatment of fruit flies until then, due to their high efficacy and low cost [70]. Regarding citrus, studies on the efficacy of these halogenated fumigants as stand-alone postharvest treatments for fruit flies have mostly been carried out on species that do not occur in South Africa: *Anastrepha* spp. [71,72] and *Bactrocera tryoni* (Froggatt) [73]. Information on the efficacy of these fumigants on fruit fly pests of citrus in South Africa are either available from other fruit commodities [74–76] or in containers [77–79]. Methyl bromide doses found to be effective for *C. capitata* and *B. dorsalis* varied between 16 and 64 g/m^3 for about 0.5–4.0 h, depending on species and temperatures used during fumigation [74–80]. There is currently a recommended dosage rate of 32 kg/m^3 applied for 0.5 h for treatment of *C. capitata* in citrus in the USA [53] but this is only permitted for movement of fruit between states.

Regarding *T. leucotreta* in citrus, Myburgh [13] conducted preliminary trials with methyl bromide fumigation, using dosages of between 4 and 16 g/m^3. Only the highest dosage, applied for 2.5 h and at 24 °C or higher, resulted in complete mortality of larvae. This treatment was never used commercially for *T. leucotreta* and it is, in any case, no longer an option. Methyl bromide was listed as an ozone-depleting substance under the Montreal Protocol of the United Nations Environment Programme (UNEP) in 1992 [81]. Its use has rapidly decreased since then, and the replacement of methyl bromide has been ongoing worldwide [82]. Regarding the other halogenated fumigant, ethylene dibromide, Schwartz and Milne [83] tested it against *T. leucotreta* eggs, larvae and pupae at rates from 4 to 20 mg/L for 2 h. Eggs were surprisingly resistant to the treatment, particularly when fresh, with almost 5% egg hatch, even at the highest dose rate. Larvae were more susceptible, with complete mortality of all larval stages at ethylene dibromide concentrations of 12 mg/L for 2 h, when tested in an artificial diet. When tested in fruit, complete mortality was only recorded at 20 mg/L. Pupae were once again more tolerant, but this was practically irrelevant, as *T. leucotreta* does not pupate in citrus fruit. As with methyl bromide, the use of ethylene dibromide is now banned throughout most of the world [70].

Alternative fumigants for treatment of quarantine insect pests have been sought prior to and after the reduction or discontinuation of the use of methyl bromide [84]. For fruit flies in citrus, the alternative fumigants explored as stand-alone postharvest treatments were phosphine [85–87] and carbonyl sulphide [88]. With phosphine, the drawbacks listed include its slow activity and therefore long duration of treatment, insect resistance, flammability and corrosion of metal tubing [84]. The slow activity of fruit fly control using phosphine was demonstrated in studies showing that fumigation times ranged from 2 to

7 d at concentrations varying from 0.25 to $\geq$1.13 g/m^3 for effective kill [85,86]. These are definitely longer fumigation periods than those required with methyl bromide. This treatment duration might not be ideal but is still a better option than cold disinfestation treatments for cold sensitive citrus cultivars. There is no available information to date on effects of phosphine as a stand-alone postharvest treatment on fruit fly pests of citrus in South Africa and on *T. leucotreta*. Investigation on the efficacy of this treatment for the latter pests is therefore warranted. Carbonyl sulphide was evaluated on *C. capitata* in lemon [88]. Similar to the findings with phosphine, long treatment durations, possibly more than 8 h, were required [88]. An important drawback identified with carbonyl sulphide is the disagreeable odor of citrus fruit after fumigation, which persisted for days. Further exploration of the use of this fumigant for postharvest treatment of quarantine insect pests of citrus would perhaps not be carried out because of this drawback.

Regarding *T. leucotreta*, the only alternative fumigant tested as a postharvest treatment was carbon dioxide (CO_2) [89]. In their study, Grout and Stoltz [89] subjected third and fifth instars of *T. leucotreta* in oranges to CO_2 at a range of concentrations from 35 to 70% for 12–24 h, at a temperature of 25 °C. Results between batches of *T. leucotreta* were extremely variable. However, CO_2 at a concentration less than 70% was generally inadequate. The most effective treatment was 24 h under 70% CO_2, after which a maximum of 99.14% of fifth instars were recorded dead. However, *T. leucotreta* was never completely controlled and the variable results made the efficacy of the stand-alone fumigation treatment too unpredictable. Given that the stand-alone CO_2 treatment was not effective for *T. leucotreta*, exploration of this treatment for disinfestation of fruit flies in citrus from South Africa was not pursued.

7. Fumigation–Cold Combination

In the 1970s, combinations of the halogenated fumigants, either methyl bromide or ethylene dibromide, and cold were tested on *T. leucotreta* and on two of the fruit fly pests that occur on citrus in South Africa: *C. capitata* and *B. dorsalis*, although this was on fruit commodities other than citrus. Seo et al. [90] showed that methyl bromide fumigation prior to cold treatment was more effective for *C. capitata*. The authors found that unrefrigerated fruit (papaya and avocado) infested with either *C. capitata* or *B. dorsalis* were effectively treated when subjected to methyl bromide at 32 g/m^3 for 2–3 h, followed by cold storage at 1.7–12.8 °C for 3–11 d [90]. Regarding *T. leucotreta*, in both citrus fruit and artificial diets, Schwartz and Milne [83] reported the efficacy of a combination treatment of ethylene dibromide fumigation and cold. They concluded that ethylene dibromide fumigation alone, at acceptable dosage rates, gave unsatisfactory control and cold alone, at rates necessary for control, was detrimental to fruit quality. Treatments of fruit infested with *T. leucotreta* with ethylene dibromide fumigation, at 16 mg/L for 2 h, followed by exposure to either 4.4 °C for 18 d [83] or 11.1 °C for 21 d [91] were found to achieve complete disinfestation of fruit from larvae of the pest.

Grout and Stoltz [89] investigated something similar with CO_2 and cold. By following CO_2 fumigation with a short cold treatment, complete control of *T. leucotreta* was achieved. The efficacy of the combined treatment was reduced when it took 12 h or more to move fruit after fumigation into a cold room at 2 °C, or if the temperature was not reduced below 12 °C within 24 h after fumigation. In these cases, the benefit of the short cold treatment was lost and mortality was similar to CO_2 fumigation alone. Grout and Stoltz [89] tested a range of combinations of CO_2 fumigation and cold, but ultimately concluded that fumigation with 70% CO_2 for 24 h followed by 13 d (or 11 d in some cases) at 2 °C was successful in controlling all fifth instar *T. leucotreta* in a range of different citrus types and cultivars. They suggested that this sequential treatment could, therefore, be a promising alternative postharvest treatment for *T. leucotreta* in all citrus and that further verification trials were warranted. A synergistic effect of CO_2 fumigation and cold treatment was also found for treatment of *C. capitata* in citrus [92,93]. On *C. capitata*, CO_2 fumigation pre-empting cold treatment, as well as the reverse order of these treatments, were evaluated and found to be effective [92,93]. In the studies on *C. capitata*, CO_2 was used at a much higher rate (95% for 20 h) than that tested by Grout and Stoltz [89] on *T. leucotreta*. Cold treatment implemented after CO_2 could be shortened to 4 d at 1.5 °C [93], as opposed to a stand-alone cold treatment of 14 d at 1.5 °C. When cold treatment preceded CO_2 fumigation, 3 d at 1.5 °C was found to be effective for killing third instars of *C. capitata* [92]. Practically, as discussed by Grout and Stoltz [89], for citrus from South Africa, it would be easier to implement a treatment where CO_2 precedes cold treatment, the latter being implemented when fruit are in transit to export markets. The combined CO_2 and cold treatment proposed by Grout and Stoltz [89] on fruit flies in citrus has been shown to be effective against *C. capitata* using 70% CO_2 in air for 24 h followed by 14 d at 2 °C [94]. Differences in tolerances to the combined treatment between fruit fly species should be sought in order so that further trials are only necessary on the most tolerant species.

8. Heat Treatments

Regarding the use of heat as a postharvest treatment, a larger body of research currently exists for fruit flies than for *T. leucotreta*. Heat treatments of quarantine pests entail exposure of these insects to high temperatures over a few hours, using air (containing or saturated with water vapour) or water as carriers of heat [95]. On citrus, two types of heat treatments were tested on fruit flies, not specifically the South African pest species: vapour heat (air saturated with water vapour) and hot water immersion [96–103]. Vapour heat treatments form part of existing approved schedules for disinfestation of imported citrus from fruit fly pests by some countries such as the USA (citrus from Hawaii) and Japan (pummelo, *Citrus maxima* Merr. from Thailand) [53,104]. For *C. capitata* and *B. dorsalis* in citrus, recommended pulp temperatures at the end of the treatment are between 46 and 47 °C and the holding times for these temperatures vary between 5 and 30 min [53,104].

Preliminary studies are underway to test the efficacy of vapour heat for disinfestation of *T. leucotreta* in citrus fruit (Grout and Stoltz, unpublished data). Efficacy of temperatures up to 47 °C against third and fifth instars are being tested and thus far mortalities approaching 90% have been recorded (Grout and Stoltz, unpublished data). However, the research is ongoing.

9. Phytotoxic Risks of Postharvest Treatments

Postharvest treatments developed for citrus should, in principle, not be detrimental to the quality of the fruit. Negative impacts on external and/or internal quality of citrus have, however, been recorded for many of the postharvest treatments with incidences of damage increasing under certain conditions (treatment intensities, citrus types, citrus harvest times, etc.). Cold storage/treatment of citrus, although important at an ideal temperature range to increase shelf life of the fruit (between 5 and 10 °C), can induce chilling injury, particularly at the lower temperatures required for phytosanitary disinfestation, with white grapefruit, lemon, and Satsuma mandarins and numerous individual citrus cultivars being particularly susceptible to it [49,105,106]. Cold storage of lemons at 1 °C for 14 d, a cold treatment protocol for fruit fly disinfestation, was found to cause chilling injury with extent of injury varying depending on harvest time and degreening practices [107]. Hattingh et al. [105] demonstrated that a temperature of 2 °C for 18 d resulted in chilling injury to Nova mandarins, ranging from 0 to 18.37% and averaging 6.36%.

Irradiation, particularly at the generic minimum absorbed dose of 150 Gy recommended for treatment of fruit flies [54], can be phytotoxic to some citrus types [108,109]. Grapefruit, particularly when harvested from gibberellic acid treated trees, were detrimentally affected by irradiation at doses higher than 300 Gy [110]. Fruit peel pitting increased and flavor was affected. Patil et al. [111] found that an irradiation dose of 400 Gy had detrimental effects on quality of early season 'Rio Red' grapefruit. In South Africa, Barry et al. [65] tested irradiation dosages ranging from 0 to 900 Gy against a range of citrus cultivars. Irradiation affected internal quality aspects, e.g., the total sugar content was increased, while juice content decreased in some cultivars. Irradiation led to a drastic increase in decay of especially lemon fruit during storage, as well as a very high incidence of rind physiological disorders. Navel orange and Clementine mandarin rind quality were both negatively affected at high dosages but lemon rind was extremely sensitive even at low dosages. Off-taste also developed in the irradiated fruit as the time in storage increased (probably due to anaerobic respiration setting in). On the whole, irradiation at 300 Gy seemed to be the limit at which certain *Citrus* species could tolerate this treatment.

Fumigation using methyl bromide at recommended doses for fruit fly disinfestation was found to have negative impacts on quality of some citrus types [112]. Fumigation with phosphine delivered as a cylindered gas mixture with nitrogen, on the other hand, was found to be safer for citrus fruit quality [112].

There are contrasting reports on the impacts of quarantine heat treatments on quality of citrus, with some showing negative impacts, particularly with regard to flavor in oranges [113,114] and others showing no negative impacts at specified pulp temperatures, humidity conditions and durations [97,98,103,115]. However, commercial use of quarantine heat treatments of citrus fruit is very limited.

Given that stand-alone postharvest treatments can potentially reduce fruit quality, a systems approach, where independent treatments are implemented, both at preharvest and postharvest levels, to reduce risk of quarantine pests, would be better to provide both pest risk mitigation and preservation of fruit quality.

10. Systems Approach: A Way to Reduce Risk of Stand-Alone Postharvest Treatments

Moore et al. [116] reported the development of a systems approach for *T. leucotreta* that included several pre and postharvest components. The postharvest components were: (1) in-orchard grading out of potentially infested fruit, immediately after harvesting; (2) fruit inspections for *T. leucotreta* infestation on delivery at the packinghouse, to determine subsequent handling requirements; (3) packinghouse grading of fruit on the packing line, involving the removal of any fruit appearing to be damaged or infested; (4) inspection of a 2% fruit sample per pallet of fruit packed for export and rejection of any pallet in which live *T. leucotreta* was detected; (5) prescription of shipping condition options for each export consignment according to compliance with preceding steps of the systems approach; and (6) official phytosanitary certification of compliant consignments. Moore et al. [116] also specified maximum measurement levels (thresholds) for the postharvest inspections.

Van Klinken et al. [117] collated and analyzed 60 protocols for implementing systems approaches in international and inter-state trade, and concluded that Moore et al. [116] presented the most comprehensive quantitative analysis of the efficacy of a systems approach, using empirical data from pre and postharvest measures to estimate the proportion of fruit packed for export that could be infested. However, in a subsequent study, the systems approach was revised and further improved, based on the outcome of a validation study [105]. This was completed by correcting errors in the original version, updating parameter values and adding a component that provides for comparison with the risk mitigation provided by a stand-alone disinfestation treatment. Consequently, it was calculated that the maximum potential proportion of fruit that could be infested with live *T. leucotreta* larvae after application of the systems approach, was no greater than the proportion of fruit that could be infested after application of a Probit 9 efficacy stand-alone disinfestation treatment to fruit with a 2% pre-treatment infestation.

The post-packing handling conditions (the cold-chain during shipping and pre-shipping) prescribed within the systems approach are those reported by Moore et al. [118] and Moore et al. [17], as described in the above section on Partial Cold Treatments. The systems approach is currently accepted by the European Union as an effective alternative to a stand-alone cold disinfestation treatment and has been used for export of citrus fruit (excluding lemons and limes, as these are non-hosts for *T. leucotreta* [6]) from South Africa to the EU since 2018.

A systems approach for mitigating the risk of fruit fly pests was also approved as a treatment protocol for export of citrus from South Africa to the European Union. Similar to the *T. leucotreta* systems approach, the systems approach for fruit flies also includes several pre and postharvest components. Most of the postharvest components currently being used in the fruit fly systems approach are similar to those in the *T. leucotreta* systems approach.

11. Future Perspectives

None of the postharvest treatments described in this paper have yet been exhaustively explored for phytosanitary disinfestation of citrus fruit from South Africa for *T. leucotreta* and fruit flies, including cold treatments. For example, there may well be warmer temperatures that could be used as stand-alone disinfestation treatments, than those tested to date, albeit for longer durations. The benefit would be reduced chilling injury and thus inclusion of cultivars that would normally be considered too chilling injury sensitive for a stand-alone cold disinfestation treatment. Combination treatments, such as those using fumigation or irradiation followed by a less severe (temperature and duration) cold treatment than would be used as a stand-alone treatment, have only been experimentally investigated but not yet employed in commercial practice. Experimentation with vapour heat treatments is only just underway. There may also be more novel approaches that warrant investigation, such as the inclusion of botanical toxicants such as natural pyrethrum in the packinghouse dip tank [119]. Furthermore, as is the case with irradiation, there should be general acceptance that mortality of the target organism should not be the only acceptable outcome of the treatment. Greater discernment must be exercised by importing countries, such as the determination of the probability of a consignment of fruit being free of a mating pair, as proposed by Follett and McQuate [120] and as demonstrated by Hattingh et al. [105] for *T. leucotreta*.

However, due to the stated shortcomings and risks of many of the existing and potential stand-alone treatments, and even some of the combination treatments, there should be a strong preference for the development and improvement of multi-tiered systems approaches for managing *T. leucotreta* and fruit flies. Although the multi-tiered systems approach for *T. leucotreta* has been scientifically and statistically demonstrated to exercise a high level of efficacy in disinfestation of citrus fruit [105,116], four years of commercial experience with the system has highlighted where further improvements can be made. As citrus is generally less susceptible to infestation by fruit flies and as fruit flies are more cold sensitive than *T. leucotreta*, the systems approach employed for *T. leucotreta* is likely to be even more effective against fruit flies.

Finally, an area of research that is currently receiving more attention than in the past is that of postharvest detection of infested fruit, as opposed to blind or blanket postharvest disinfestation. This involves technologies such as X-ray (particularly microfocus X-ray tomography), gas chromatography mass spectrometry (GCMS), selected ion flow tube mass spectrometry (SIFT-MS), sniffer dogs and sniffer wasps. All these technologies have been or are currently being investigated specifically for postharvest detection of *T. leucotreta* or fruit fly infested citrus fruit in South Africa, e.g., [121,122]. Demonstration of sufficiently accurate detectability of infested fruit with any of these technologies may lead to a system for removal of such fruit in the packinghouse, disqualification of an orchard or consignment of fruit for a sensitive market, or more targeted application of a disinfestation treatment.

In conclusion, phytosanitary protection is achieved through regulatory plant protection, which aims to safeguard agricultural crops from such pests and diseases [123]. Such regulations and programmes must continually be in place to prevent pests from being transported along with these commodities [123]. As global trade of susceptible fresh commodities increases, so does the risk of spread of pests and diseases [124]. The economic costs associated with alien species have been estimated to amount to about 5% of the world gross national product (GNP), underscoring the importance of phytosanitary protection [125]. Consequently, research into and development of novel, effective and safe (specifically to the commodity) postharvest risk mitigation measures will continue to be prioritized. South Africa is the second largest exporter of citrus worldwide, after Spain, and the largest shipper of citrus worldwide [1]. Due to the value of these export markets to South Africa, development and improvement of such measures for pests that can potentially infest South African citrus, such as *T. leucotreta*, *C. capitata*, *C. rosa* and *B. dorsalis*, will remain at the top of the southern African citrus industry's research agenda.

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