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Efficacy and Phytotoxicity of Sulfur Dioxide Fumigation for Postharvest Control of Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), on Select Fresh Fruit and Vegetables

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Abstract: Sulfur dioxide (SO_2) fumigation was evaluated for efficacy against western flower thrips (*Frankliniella occidentalis*) and phytotoxicity to four select fresh fruits and vegetables. Western flower thrips were found to be very susceptible to SO_2 fumigation. Fumigations with 0.3 and 0.5% SO_2 for 60 and 30 min, respectively, at a low temperature of 5 °C achieved 100% thrips mortality. Broccoli, bell peppers, apples, and navel oranges with thrips were subjected to 30 min fumigation with 0.3–0.5% SO_2 to verify efficacy and determine potential phytotoxicity. The fumigation resulted in complete control of thrips. Its effects on visual quality of fresh produce varied. The fumigation caused severe discoloration of broccoli. However, the treatment did not have significant effects on the color of other products. No negative impact on visual appearance of bell peppers and navel oranges was observed. However, it caused darkened lenticels on green apples and, therefore, may potentially degrade apple postharvest quality. The lack of phytotoxicity of SO_2 fumigation is likely due to well-developed wax layers on those fresh products. The results of the study suggest that SO_2 fumigation has good potential to be used safely and effectively against sensitive pests on select fresh fruit and vegetables including peppers and citrus fruits.

Keywords: postharvest pest control; postharvest quality; broccoli; bell pepper; apple; navel orange

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1. Introduction

There is a need for more alternative fumigants to replace methyl bromide which has been phased out globally due to its depleting effects on atmospheric ozone. Currently, phosphine and sulfuryl fluoride are the main alternative fumigants, and both have major shortcomings. Phosphine fumigation acts slowly and has long treatment times. Some insects either developed resistance or are naturally tolerant to phosphine fumigation [1,2]. Oxygenated phosphine fumigation is more effective than regular phosphine fumigation [3]. But there have been no commercial applications. Sulfuryl fluoride fumigation is not effective against insect eggs, and it is also phytotoxic to fresh produce [4,5]. Recently, there has been progress in developing safe ethyl formate fumigation treatments for postharvest pest control on selected fresh products such as navel oranges [6,7]. But usage of ethyl formate is likely very limited to cause phytotoxicity to fresh products [8,9]. Although nitric oxide fumigation is effective for postharvest pest control, its prospect for commercial application is uncertain due to its complex and stringent fumigation procedures as well as higher costs [10]. Therefore, new alternative fumigants are urgently needed to meet the needs of postharvest pest control.

Sulfur dioxide (SO_2) is widely used as a preservative to control microbes on fresh and stored products [11–13]. It has also been used as a pesticide since the early 1900s [14]. However, it is not commonly used to control postharvest pests even though there have been several studies showing potential of SO_2 fumigation for postharvest pest control [15–20].

One-hour short fumigation with 0.16% SO_2 results in 100% mortality of third instar omnivorous leafroller larvae [15]. Fumigation with 1% SO_2 combined with 6% CO_2 at 19.5 °C controls black widow spiders on table grapes [17]. Fumigations of 30 min at 20 °C with 0.25, 0.5, and 0.75% SO_2 result in 18, 73, and 100% mortalities of grape mealybug crawlers [16]. SO_2 fumigations at 400 and 500 ppm result in 89.8% and 95.8% mortality of grape mealybug eggs, respectively, and 100% mortality for nymphs and adults in 24 h at 2 °C [18]. Three-hour fumigation with 2% SO_2 was also demonstrated to be effective in controlling navel orangeworm on pistachios [19]. Spotted wing drosophila and blueberry maggot were successfully controlled with a 3-day fumigation with 0.05% SO_2 at 22 °C followed by 6-day cold storage at −0.5 °C [20]. All these results indicate that SO_2 has potential to be used as a fumigant for postharvest pest control.

The main challenge for SO_2 fumigation to be used for postharvest pest control on fresh produce is its phytotoxicity. However, for any fumigant, phytotoxicity depends on the severity of the treatment and relative tolerance of the fresh produce to be treated. For SO_2 fumigation, table grapes have been reported to tolerate a treatment that controls mealybugs [18]. There is a lack of studies on susceptibility of different pests as well as tolerance of different fresh products to SO_2 fumigation. In this study, SO_2 fumigation was evaluated for control of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and effects on postharvest quality of four selected fresh fruits and vegetables: apples, navel oranges, broccoli, and bell peppers, to determine its potential for postharvest pest control on fresh produce.

Western flower thrips is a native insect of North America and major pest of a wide variety of outdoor crops as well as greenhouse vegetables and nursery plants. Thrips feed on leaves and buds, causing leaf scarring, wrinkling, and deformation. They also feed on flowers where they consume pollen and can cause blossom drop or scar development on fruit. WFT is also an important vector of many plant virus diseases including impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV) [21]. It is a quarantined pest on some overseas markets such as Taiwan and Australia [22] and, therefore, affects export of US fresh fruit and vegetables. Peppers are particularly susceptible to WFT. Western flower thrips are attracted to the blossoms of apples as well as orchard cover crops and weeds. The primary damage is from egg-laying punctures in newly formed fruit, which typically occur before petal fall. Although WFT is not a major pest of navel orange, navel orange can provide overwintering sites for WFT and, therefore, may transport WFT overseas on exported citrus [6].

Low-temperature phosphine fumigation is effective and safe to control WFT on fresh produce [23] and has been used to control WFT on internationally traded fresh products. An ultralow oxygen (ULO) treatment was also developed to control WFT on harvest lettuce [24] but has not been used commercially. Nitric oxide fumigation is also effective against WFT on fresh products without negative effects on postharvest quality [25]. Since SO_2 is a generally recognized as safe (GRAS) compound, successful control of WFT with SO_2 fumigation on fruit and vegetables would provide a greener and better solution to the pest problem on certain fresh produce.

2. Materials and Methods

2.1. Insect and Materials

Western flower thrips were reared on lettuce plants through natural infestation in a greenhouse under natural lighting. For fumigation tests, larvae and adults were collected in small plastic vials (3 cm in diameter by 7 cm in height) each containing a piece of lettuce leaf using a vacuum powered aspirator (ca.20/vial). The vials with thrips were sealed with screened lids and kept at 2 °C in a refrigerator before being used in fumigation tests. A mix of 20% SO_2 with air in a compressed cylinder from Praxair (Danbury, CT, USA) was used for fumigation. The SO_2 gas was released and stored in a foil bag and gas samples were taken from the foil bag with a gastight syringe for fumigation tests. Broccoli crowns, bell peppers (green and red), apples (green and yellow), and navel oranges were obtained from local supermarkets and stored in a refrigerator before being used in fumigation tests.

2.2. SO_2 Fumigation of Western Flower Thrips

Western flower thrips were fumigated with SO_2 at different concentrations for 30 and 60 min at 5 °C in 1.9 L jars to determine effective treatments. The lid of each jar was modified to have two ports equipped with stopcocks and jars were sealed using silicone ring gaskets between lids and jar rims. In each test, 3 vials each with ca. 20 thrips were set up in each jar and subjected to a SO_2 fumigation treatment. For 30 min fumigations, SO_2 doses were 0.1, 0.2, 0.3%, and 0.5%. For 60 min fumigations, SO_2 doses were 0.1, 0.2, and 0.3%. SO_2 concentrations in all jars were measured at the end of each fumigation using a multi-gas meter equipped with a 0–5000 ppm high-concentration SO_2 sensor (Kane 900, Kane International Ltd., Welwyn Garden City, Hertfordshire, UK). SO_2 levels were measured by connecting an inlet and an outlet of the instrument to the two ports on the lid of a jar. Untreated thrips were used as controls in each test. At the end of fumigation, treatment jars were ventilated in a fume hood to terminate treatments and thrips in vials were then held at 5 °C overnight before being scored for mortality under a microscope. Thrips that were motionless in response to probes with a soft brush were classified as dead. Moribund thrips that could move legs but were not able to walk were also classified as dead.

2.3. SO_2 Fumigation of Fresh Produce

Broccoli, bell peppers (green and red), apples (yellow and green), and navel oranges from local supermarkets were fumigated with 0.3–0.5% SO_2 for 30 min together with WFT in vials in a 19.8 L plastic cylinder chamber to verify efficacy against WFT and determine effects on postharvest quality of fumigated produce. The chamber was described previously [19] and has an air blower fan (SparkFun Blower–Squirrel Cage, Sparkfun Electronics, Niwot, CO, USA) connected to a vertically positioned tube to circulate air in the chamber. Apples and navel oranges were fumigated in the same chamber. In most tests, 3 yellow and 3 green apples and 3 navel oranges were fumigated in one test. The same numbers of untreated fruits were used as controls. Green bell peppers and red bell peppers were fumigated together in the same chamber. In each test, 3 green and 3 red bell peppers were fumigated, and the same numbers of green and red bell peppers were used as controls. The fumigation test was replicated 3 times. In each fumigation test, 5–6 vials of WFT (about 20/vial) were placed in the chamber together with fresh produce and 3–5 vials of WFT were used as controls. A total of 713 WFT were fumigated with fresh produce and 509 WFT were used as controls. Broccoli crowns were fumigated separately without WFT, and the fumigation was not replicated due to severe discoloration.

All fumigations were conducted in a walk-in cooler at 5 °C. The air blower in the fumigation chamber was turned on for the 30 min duration of fumigation. After initial SO_2 injection, the SO_2 level in the chamber was measured immediately. Depending on different products, additional injections of SO_2 were made based on SO_2 level declines to maintain SO_2 levels in the range of 0.3 to 05%. For broccoli, considerable sorption of SO_2 occurred and 7–8 additional injections of 200 mL 20% SO_2 were made after an initial injection of 800 mL of 20% SO_2 to maintain SO_2 concentrations above 0.3%. For all other products, SO_2 levels stayed relatively stable. The initial injections of 500–800 mL of 20% SO_2 resulted in SO_2 levels of 0.3–0.5% throughout the 30 min fumigation. A final SO_2 level was measured before the end of each fumigation. To terminate a SO_2 fumigation, the chamber was moved into a fume hood, opened, and ventilated for at least 20 min. WFT in vials were retrieved and held at 5 °C overnight before being scored for mortality. After fumigation, fumigated products and untreated controls were stored in 8 L plastic containers with small holes for ventilation and held at 5 °C in the walk-in cooler until being evaluated for visual quality. Broccoli and bell peppers were evaluated one week after fumigation. Apples and oranges were evaluated two weeks after fumigation due to their longer storage life than vegetables.

For visual quality evaluation, fruit and vegetables from the treatment and the control were inspected for any injuries and color differences. Fumigated products were photographed side by side with controls for visual comparisons. A spectrophotometer (CM-700d, Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) was then used measure color parameters *L**, *a**, and *b**, representing brightness, redness–greenness, and yellowness–blueness of color, respectively. Each fruit and vegetable was measured three times at different positions and the average values were used in analysis.

2.4. Data Analysis

Mortality rates of WFT among different treatments were transformed by arcsine$\sqrt{x}$ prior to one-way analysis of variance (ANOVA) and Tukey's HSD multiple range test. For color measurements of vegetables and fruits, the average values of *L**, *a**, and *b** for each individual product were calculated. For each product, average values of *L**, *a**, and *b** were calculated for the treatment and the control and they were compared between the treatment and the control using one-way ANOVA. All statistical analyses were conducted using JMP Statistical Discovery Software [26].

3. Results

SO_2 fumigation was very effective against WFT, and complete controls were achieved in 30- and 60-min fumigations with 0.5% and 0.3% SO_2, respectively (Table 1). At 0.1% concentration, 30- and 60-min fumigations resulted in 14.5% and 93% mortalities, respectively, indicating importance of treatment time. At a 0.2% SO_2 level, 30 min fumigation resulted in over 94% mortality. There were significant differences among treatments for both 30- and 60-min fumigation tests. Mortalities in the controls were 18.95% and 15.2% for 30- and 60-min fumigations, respectively (Table 1).

Table 1. Effects of 30 and 60 min SO_2 fumigations at 5°C on mortality of western flower thrips.

Time (min)	SO_2 (%) Start	SO_2 (%) End	Rep	Total	Mortality (%) Mean ± SE	ANOVA
30	0.1	0.081	9	166	38.62 ± 7.27 b	df = 4, 40
	0.2	0.176	9	164	94.67 ± 2.16 a	F = 53.3264
	0.3	0.263	9	165	98.86 ± 0.75 a	P < 0.0001
	0.5	0.432	9	179	100 a	
	Control		9	157	18.95 ± 9.55 c	
60	0.1	0.074	13	258	87.12 ± 4.13 b	df = 3, 49
	0.2	0.168	13	272	99.73 ± 0.28 a	F = 128.6937
	0.3	0.259	13	261	100 a	P < 0.0001
	Control		14	260	15.20 ± 3.89 c	

Mortality data were transformed by arcsine$\sqrt{x}$ before one-way ANOVA. For each treatment time, mortalities among different treatments followed by the same letter were not significantly different based on Tukey's HSD multiple range test at p = 0.05 [26].

Fumigations of fresh produce resulted in 100% mortality of 713 fumigated WFT as compared with <30% mortality in controls. There were large differences among the four fresh products in susceptibility to injuries by SO_2 fumigation. SO_2 fumigation caused severe discoloration of broccoli and rendered it unmarketable (Figure 1). For yellow apples, navel oranges, and bell peppers, there were no visible stains or discoloration on SO_2-fumigated products as compared with controls. For green apples, however, there were darkened lenticels on cuticles of fumigated apples which were absent on control apples (Figure 1).

There was no significant difference in *L**, *a**, or *b** values between SO_2 fumigation and the control for any product except a significantly higher *a** value for SO_2 fumigation (−10.20) than for the control (−10.87) for green apples (Table 2). When *L**, *a**, and *b** values were converted to color appearances using an online color-converting tool, there were barely any noticeable color differences between SO_2 fumigation and control for any product (Figure 2). Therefore, SO_2 fumigation for control of western flower thrips was safe for postharvest visual quality of yellow apples, bell peppers, and navel oranges. SO_2 fumigation accelerated darkening of lenticels on green apples and may degrade overall visual quality.

Figure 1. Appearance of broccoli, green and red bell peppers, green and yellow apples, and navel oranges from 30-min fumigation treatment with 0.3–0.5% SO2 at 5 °C (T) and unfumigated controls (C) at one week (broccoli and peppers) and two weeks (apples and navel oranges) after fumigation.

Table 2. Effects of 30-min fumigations with 0.3–0.5% SO_2 on color parameters of apples and navel oranges at 14 days after fumigation and of bell peppers at 7 days after fumigation.

Product	Treatment	Rep	*L**	*a**	*b**
Green apple	SO_2	8	62.02 ± 1.24	−10.20 ± 0.24	41.6 ± 1.05
	Control	8	60.93 ± 0.85	−10.87 ± 0.15	41.90 ± 1.28
	ANOVA	*F*=	0.5190	5.5648	0.0281
	df = 1, 14	*P*=	0.4831	0.0334	0.6693
Yellow apple	SO_2	8	74.26 ± 0.87	−3.08 ± 0.71	47.32 ± 0.69
	Control	8	74.06 ± 0.72	−3.15 ± 0.50	47.43 ± 0.73
	ANOVA	*F*=	0.0311	0.0055	0.0125
	df = 1, 14	*P*=	0.8625	0.9418	0.9126
Navel orange	SO_2	12	65.93 ± 0.41	33.50 ± 0.51	64.09 ± 1.12
	Control	12	64.57 ± 1.70	32.74 ± 0.78	65.40 ± 0.69
	ANOVA	*F*=	0.5989	0.6584	0.9856
	df = 1, 22	*P*=	0.4473	0.4258	0.3316
Green pepper	SO_2	9	37.16 ± 0.65	−8.90 ± 0.18	22.05 ± 0.99
	Control	9	37.16 ± 0.76	−8.75 ± 0.23	21.10 ± 1.24
	ANOVA	*F*=	0.0000	0.2457	0.3631
	df = 1, 16	*P*=	0.9977	0.6268	0.5553
	SO_2	9	32.64 ± 0.88	30.57 ± 1.23	17.23 ± 1.03
Red pepper	Control	9	33.44 ± 0.72	31.26 ± 1.21	17.26 ± 1.12
	ANOVA	*F*=	0.4904	0.1592	0.0004
	df = 1, 16	*P*=	0.4938	0.6952	0.9837

The effect test in one-way ANOVA was used to determine the difference in *L** (brightness), *a** (redness–greenness), and *b** (yellowness–blueness) between SO_2 fumigation and the control for each product. The two values of each parameter for each product were significantly different if $p < 0.05$ [26].

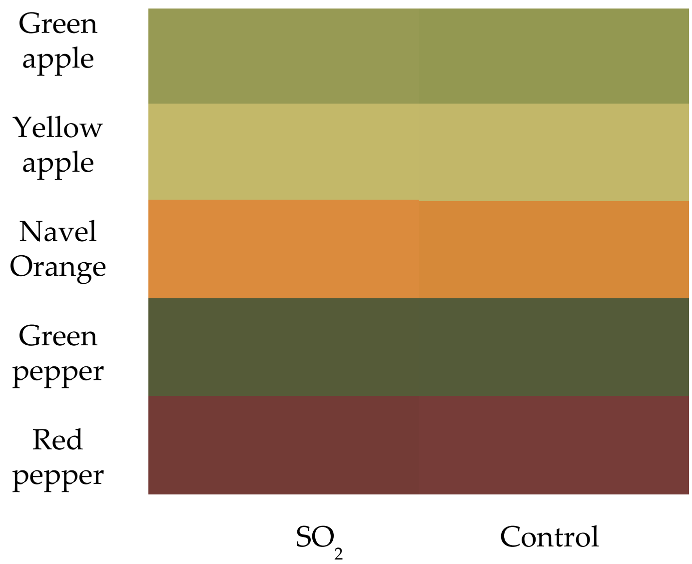

Figure 2. Color appearances of green apples, yellow apples, navel oranges, green peppers, and red peppers from SO_2 fumigations and controls based on *L**, *a**, and *b** values in Table 2 using an online color converter (https://www.nixsensor.com/free-color-converter/, accessed on 12 February 2024).

4. Discussions

The complete control of WFT in 30- and 60-min short fumigations with 0.5 and 0.3% SO_2, respectively, indicated that SO_2 fumigation was very effective against WFT. The high susceptibility of WFT to SO_2 fumigation is consistent with other insects of fresh produce. A 30 min fumigation with 0.75% SO_2 was reported to kill 100% grape mealybug crawlers at 20 °C [17]. A one-hour fumigation with 0.16% SO_2 was reported to kill 100% of third instar omnivorous leafroller larvae at 22 °C [15]. In the current study, fumigations with 0.3% and 0.5% SO_2 killed 100% of WFT in 60 and 30 min, respectively, at a much lower temperature of 5 °C. This indicated that SO_2 fumigation can be used to control WFT on harvested fresh produce during postharvest cold storage without the need to raise produce temperature. In fact, reducing temperature also reduces SO_2 sorption on stored pistachios [19]. Therefore, SO_2 is better suited for fumigation at lower temperatures than at higher temperatures.

In fumigations of fresh produce, the complete control of WFT and the lack of negative impact on visual quality of bell peppers and navel oranges showed that SO_2 fumigations at 0.3–0.5% concentrations at 5 °C have potential to be used safely on select fresh fruits and vegetables to control WFT. The 30-min SO_2 fumigation treatment did not cause significant changes of any of *L**, *a**, and *b** color parameters except a small but significant increase in *a** value from −10.87 to −10.20 for green apples. Corresponding to the difference in *a** value, SO_2-fumigated green apples had darkened lenticels which were absent on unfumigated green apples as controls (Figure 1). Tiny and discolored spots such as discolored lenticels need careful observation to detect. Even though SO_2 fumigations did not cause darkened lenticels on yellow apples, it is reasonable to assume that the same SO_2 fumigation could cause darkened lenticels on yellow apples with different postharvest physiological conditions.

In comparison with other fresh products, broccoli is very susceptible to injuries by SO_2 fumigation as indicated by color bleaching. Corresponding to the discoloration was high sorption of SO_2 on broccoli since multiple injections of SO_2 were needed to maintain SO_2 concentration above the 0.3% level. In comparison, there was very low SO_2 sorption on other products indicated by stable SO_2 levels during fumigation and the absence of phytotoxicity. Given the negative impact on broccoli visual quality and darkened lenticels on green apples, a SO_2 fumigation treatment needs to be tested on each fresh product to determine whether the treatment can be used safely for postharvest pest control.

The rationale of using a short 30-min fumigation is to reduce sorption of SO_2 on fresh products and, thereby, reduce the risk of phytotoxicity. WFT is an external pest and does not require penetration of SO_2 into plant tissue to control. Therefore, short treatments are appropriate. For an internal pest of fresh produce, a much longer SO_2 fumigation will likely be required, and a longer SO_2 fumigation treatment likely has a higher risk of phytotoxicity to fresh products.

Thrips include many important pest species [27]. In addition to WFT, other important thrips pests include bean thrips (*Caliothrips fasciatus* (Pergande)) and citrus thrips (*Scirtothrips citri* (Moulton)) which are found on navel oranges. Bean thrips are also quarantined on exported citrus fruit to Australia [22]. It is likely that SO_2 fumigation treatment for control of WFT will also be effective against other thrips on citrus fruit. Many other insects may also be as susceptible to SO_2 fumigation as WFT. Therefore, SO_2 fumigation for WFT control may also be used safely and effectively against those pests on select fresh produce. The present study opened more opportunities to further expand potential applications of SO_2 fumigation for postharvest pest control on fresh produce.

Fresh fruit and vegetables typically have a wax layer on cuticles as a barrier membrane to reduce water loss and protect from photoirradiation. It is likely that cuticular wax layers play a critical role in protecting fresh produce from injuries by SO_2 fumigation. Cuticular waxes have considerably large variations in composition, morphology, and quantity among species and are affected by biochemical pathways and environmental factors such as temperature, irradiation, humidity, and water stress [28]. Broccoli has cuticular wax [29]. However, SO_2 fumigation for WFT control caused severe injuries to broccoli as compared with no injury or very minor injuries to the other three fresh products. As compared with the smooth cuticle of other products, broccoli florets are characterized by rugged surfaces. It is likely that broccoli florets lack a uniform wax layer as an effective barrier to SO_2 gas. From the results of different responses of the four fresh products to SO_2 fumigation, fresh products with smooth waxy surfaces are likely more tolerant to SO_2 fumigation.

Lenticels on apples are formed mainly by gradual disintegration of stomata during fruit development or by removal of trichomes in young fruit [30]. Lenticels have open and closed types, and open lenticels close over time during postharvest storage due to continued synthesis of crystalline wax [31]. SO_2 fumigation has been used to detect open lenticels as SO_2 penetrates open lenticels, oxidizes exposed tissues, and forms brown spots [31]. Therefore, the darkened lenticels on fumigated green apples were open lenticels damaged by SO_2 fumigation. Safety of SO_2 fumigation for apples may depend on the presence of open lenticels and they can be detected using SO_2 fumigation.

Currently, only pure phosphine and ethyl formate are alternative fumigants to control postharvest pests on fresh products at low temperatures. For controlling thrips, the 30-min duration of SO_2 fumigation is comparable to 1 h ethyl formate fumigations [7] but much shorter than the 18 h phosphine fumigation needed to control WFT [23]. In comparison with ethyl formate fumigation, SO_2 fumigation may have an advantage of easier operation since ethyl formate needs be heated to vaporize at low temperatures [7]. There is not enough information to compare their relative safety for different fresh fruits and vegetables. However, SO_2 fumigation also has disadvantages. Like the corrosion risk of phosphine to copper, SO_2 is known to be aggressive to metals as SO_2 reacts with moisture to form sulfuric acid which is corrosive. Therefore, special modifications to fumigation enclosures may be needed to mitigate the potential corrosion risk in practical applications of SO_2 fumigation for postharvest pest control.

SO_2 has a long history of being used as a pesticide. But it has only been studied to a very limited extent for postharvest pest control. This study represented meaningful progress in expanding applications of SO_2 fumigation in postharvest pest control on fresh produce. Given that sulfur is a macronutrient and SO_2 is a GRAS compound, SO_2 fumigation has advantages in food safety over most other fumigants and more research efforts on SO_2 fumigation are warranted. For fresh fruit and vegetables, efforts are needed to determine effective treatments against various postharvest pests and effects on postharvest quality and storage/shelf-life of various fresh fruits and vegetables. Efforts are also needed to develop commercial-scale treatment protocols for practical applications.

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