The Control Efficacy of Sodium Hypochlorite against Violet Root Rot Caused by *Helicobasidium mompa* in Apple

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Our study was carried out to determine the control efficacy of sodium hypochlorite (NaOCl) for violet root rot caused by *Helicobasidium mompa* in apple. The experiment was conducted in the farm located at Chungbuk province in South Korea from 2014 to 2016. When infected apple trees were treated at least two or three times with 31.25 and 62.5 ml/l available chlorine content in NaOCl, it greatly increased the rooting of rootstock, and restored the tree crown density by 44.4-60.5%. In addition, the number of commercial fruit setting was increased by 54.3-64.5%, and the total starch content in shoots was significantly higher than other non-treated apple trees. However, the untreated disease control and thiophanate-methyl WP treated trees showed the symptom of dieback. Therefore, our results indicate that the drenching treatment of NaOCl with 31.25-62.5 ml/l available chlorine content more than two times from late fall to early spring could effectively control the violet root rot and recover tree vigor up to 60%.

**Keywords**: apple dieback, sodium hypochlorite, violet root rot

Apple is one of the major fruit crops in South Korea and its production is worth about $1.5 billion per year (http://www.krei.re.kr). Recently, climate changes and different cultivation environment have greatly led to the dieback problem of apple trees caused by soil-borne disease pathogens (Lee et al., 2016, 2020). Due to this problem, the economic loss of apple growers in South Korea is estimated to be around $2 million annually (Lee et al., 2020).

The violet root rot caused by *Helicobasidium mompa* is one of the most serious soil-borne diseases in apple trees (Korean Society of Plant Pathology, 2009; Lee et al., 2006; Rural Development Administration, 1993). The infected trees show drastically weakened leaf growth, poor growth of shoots, vacant branches, and early leaf yellowing and defoliation, which leads to reduced fruit yield and lowered quality. When trees are severely infected, the whole trees show a decline and all dieback within 2-3 years. Furthermore, re-occurrence of the disease symptoms is highly possible even after supplementary planting with the nursery trees. It is more likely that the fields are not recommended to be used again for apple cultivation (Kim et al., 1995; Lee, 2002; Lee et al., 1995, 2016, 2020).

Meanwhile, when symptoms are already shown and the infection spreads, the violet root rot symptoms would be too severe to control the infected trees. Growers would finally lose all the trees as a result from the dieback (Lee et al., 2009). Furthermore, the diseases could be spread through the soil continuously if the infected roots are not completely removed (Lee et al., 2016). This would be another big economic loss to the apple growers.

Currently, only two active ingredients, such as tolclofos-methyl and thiophanate-methyl WP, have been registered as fungicides to control violet root rot in apple tree (http://
www.koreacpa.org). These fungicides have been used in most of farms where trees suffer from dieback, but the fungicides are not effective to control the disease. Thus, the annually repetitive supplementary planting of the nursery trees is the only alternative way to control the disease, which causes high economic losses every year (Lee et al., 2019).

Sodium hypochlorite (NaOCl), a strong chemical disinfectant, could be used as an effective alternative for controlling soil-borne diseases (Lee, 2018). However, NaOCl is registered to apply in agricultural fields in South Korea, while it has been registered as a crop protection agent (U.S. Environmental Protection Agency, 1991) in the USA. It is necessary to develop alternative control agents and methods to control soil-borne diseases that cause dieback in other fruit trees as well as apple trees.

Recently, NaOCl was tested in vitro as a chemical control agent for violet root rot. The result showed the high level of colony inhibition against three different field isolates of H. mompa (Lee, 2018; Lee et al., 2019).

Therefore, the purposes of our study were to confirm the possibility for both efficacy of disease control and potential recovery of tree vigor by the drenching treatments of 31.25 and 62.5 ml/l available chlorine contents in NaOCl to apple trees infected by H. mompa.

### Materials and Methods

#### Selection of infected apple trees

To confirm the control efficacy of NaOCl against violet root rot caused by H. mompa, we performed field trials in the apple farm located at Jangyeon, Goesan, Chungbuk from 2014 to 2016. From the fruiting body of H. mompa occurred to the apple farm, we confirmed the hypae characteristics of the pathogen using optical microscope and digital camera (Leica DE/DM2000 and Leica microsystems DE/DC180, Wetzlar, Germany). The number of accumulated dieback trees was about 120 among 3,850 in the farm. The tested apple cultivar was ‘Sansa’ with M.26 own-root stock of 6-8 years old. The farm environment showed an altitude of ca. 201 m, a western aspect with a gradient of 5-10%, and good vertical drainage with silt loam of 15-30%. The selection of infected trees for treatment was determined based on tree crown density which was represented by multiplying the relative ratio of leaf size with three indices: five (ca. 6 × 8 cm), three (ca. 4 × 6 cm), and one (ca. 2 × 4 cm), and foliage amount (Table 1).

#### Tested chemicals

The sodium hypochlorite (NaOCl, available chlorine content 1,000 ml/l, Junsei, Tokyo, Japan) was divided to 31.25 and 62.5 ml/l, respectively. Each diluted solution was treated once in 2014 (May 26), twice in 2015 (March 27 and May 21), and thrice in 2015-2016 (November 12, 2015, March 22, and May 19, 2016) and drenched more than 10 l per tree on the grafting region. All treatment plots were conducted by repeating three times with completely randomized design.

For the fungicide control, thiophanate-methyl (WP, a.i. 70%), was drenched approximately 40 l per tree following the instructions of The Korea Crop Protection Association (http://www.koreacpa.org).

#### Experimental design

In early August, when the fruit harvest began, the measurement of crown density of the treated trees was conducted by examining the distribution rate of leaf size and foliage amount. The data were compared with the healthy trees. In each mid-October from 2014 to 2016, the number of dieback trees was surveyed, and the shoot length for each treatment was measured by categorizing them as short (5 cm or less), intermediate (5-30 cm or less), and long (above 30 cm).

At the same time, the characteristics of apple fruit was examined in 10 fruits per repetition. The total soluble solid was calculated using the Refractometer (PAL-1, Atago, Tokyo, Japan) and the acidity was titrated to pH 8.1 with 0.1 N NaOH, and then converted to the corresponding amount of apple acid. The firmness was converted to Newton (N, N = measurement value × 1.995156 × 9.8065) using the Ø 8 mm Fruit Hardness Tester (FHM-5, Takemura Denki Manufacture Co., Tokyo, Japan) and other analyses were followed by the Rural Development Administration’s Research and Analysis Standards (Rural Development Administration, 2012). In the mid-October, the total starch content of fruit-bearing shoots was assayed using the Table 1. The number of accumulated dieback trees affected by violet root rot post-treatment

| Treatment | Surveyed date |
|-----------|---------------|
| UDC       | Oct 30, 2014  |
|           | Oct 13, 2015  |
|           | Oct 25, 2016  |
| TM        | 0/3           |
|           | 1/3           |
| 31.25 ml/l NaOCl | 0/3  |
| 62.5 ml/l NaOCl  | 0/3  |

Number of accumulated dieback trees/Number of treatment trees diseased.

Treatment frequency of each treatment was once (May 26, 2014), twice (March 27, 2015 and May 21, 2015), and thrice (November 12, 2015, March 22, 2016 and May 19, 2016).

UDC, untreated disease control; TM, thiophanate-methyl WP fungicide.
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tal Starch Assay Kit (Megazyme, Wicklow, Ireland) and measured in 510 nm of the Spectrophotometer (Molecular Devices Spectra Max, San Jose, CA, USA), following an AOAC method 996.11 (Horwitz and Latimer, 2005).

Data analysis. The mean could be compared with the respective control using the Duncan’s test (P = 0.05) and two-way ANOVA analysis when Duncan's multiple range test showed a significant differences (P < 0.05) by using the CoStat 6400 program (CoHort Software, Monterey, CA, USA).

Results and Discussion

The number of dieback trees and the relative crown density by each treatment. The typical symptom of apple trees infected by H. mompa showed significantly reduced tree vigor, which causes poor growth of shoots, yellowing leaves, poor fruit-setting and quality, and dieback in harvest season (September to November). Moreover, the fruiting body of H. mompa with the color purple to dark brown was formed at the soil surface and root. And as reported by Lee (1995), we identified pathogens based on the brown mycelium with septa and rectangular branches (Fig. 1). It was tested whether using NaOCl as a treatment agent was effective in recovering the noticeably poor vigor of the trees and the effect of control was examined by measuring, rooting formation, tree crown density, number of dieback trees, growth of shoots, number of fruit-setting, fruit characteristics, and total starch content of the fruit-bearing shoots. We found that the fruiting body of H. mompa was destroyed by treatment with NaOCl 31.25 and 62.5 ml/l, and roots were remarkably regenerated after treatment in two to three months (Fig. 2).

The number of accumulated dieback trees after each treatment is shown in Table 1. When the first survey was conducted in 2014, dieback symptom was not observed in any tree plots. But two trees from the untreated disease

![Fig. 1. The typical symptom and sign of violet root rot caused by Helicobasidium mompa in an apple farm. (A) The poor growth with vacant branches in tree crown by decline of tree vigor (August 2, 2016). (B) Early leaf yellowing and defoliation in tree crown by decline of tree vigor (September 22, 2015). (C) The acute dieback occurred on the period of fruit harvest (October 31, 2012). (D) The fungal fruiting body occurred to grafting region (July 13, 2015). (E) The violet mycelia occurred to rootstock (August 19, 2016). (F) Brownish mycelia showing septa and rectangular branches under 400× optical microscope (May 16, 2014).](image-url)
control (UDC) plot and one tree from the plot treated with tiophanate-methyl WP fungicide (TM) showed dieback symptom after fruit harvest in 2015. Between 2015 and 2016, additionally, one tree from the UDC plot and two trees from the TM plot showed dieback symptom, respectively. However, The plots treated with NaOCl 31.25 and 62.5 ml/l showed no dieback symptom.

The relative tree crown density based on the foliage amount and distribution rate of leaf size for the infected trees before NaOCl treatments is shown in Table 2. Before each treatment, there was no significant difference among the infected trees and the relative tree crown density of infected trees compared to the healthy trees (100%), as it was 35-41%. The relative tree crown density according to treatment frequency by each available chlorine content in NaOCl is shown in Fig. 3. The field data were collected on August 6, after the once treatment. There was a significant difference in the relative tree crown density in all treatments. The treatment with NaOCl 62.5 ml/l showed the most significant increment in all treated trees. By examining the result on August 4 after two-time treatments, each NaOCl 31.25 and 62.5 ml/l treatment showed a significant difference at 44.4 and 56.4%, compared to the relative tree crown density of healthy trees. But there was no difference by available chlorine content in NaOCl. The results shown on August 8, 2016, after three-time treatments using NaOCl 31.25 and 62.5 ml/l showed a significant difference at 49.8 and 60.5%, respectively. The NaOCl 62.5 ml/l treatment was more effective than 31.25 ml/l treatment for the increase in relative tree crown density.

Therefore, our results indicate that more than two-time drenching treatments of NaOCl 31.25-62.5 ml/l, at least 10 l or more, can improve the tree crown density with increment of foliage amount and leaf size to 44.4-60.5% level, compared to those of healthy trees.

### Table 2. Healthy degree based on foliage amount and distribution rate of leaf size in tree crown for diseased trees pre-treatment

| Treatment      | Foliage amount (A) | Distribution rate of leaf size (cm) | Total | Healthy degree |
|----------------|--------------------|------------------------------------|-------|----------------|
|                | 6 × 8              | 4 × 6                              | 2 × 4 |                |
| Healthy        | 1 a                | 0.7 a                              | 0.2 c | 0.1 a          | 4.2 a | 1               |
| UDC            | 0.6 b              | 0.2 b                              | 0.5 a | 0.3 a          | 1.7 b | 0.59            |
| TM             | 0.6 b              | 0.2 b                              | 0.6 a | 0.2 a          | 1.6 b | 0.61            |
| 31.25 ml/l NaOCl| 0.5 b             | 0.2 b                              | 0.4 bc| 0.3 a          | 1.5 b | 0.65            |
| 62.5 ml/l NaOCl| 0.6 b             | 0.2 b                              | 0.5 ab| 0.3 a          | 1.6 b | 0.62            |

Mean separation by Duncan’s multiple range test at $P < 0.05$.

Total = 5 $(A \times B) + 3 (A \times C) + 1 (A \times D)$.

Healthy degree = (healthy total – each treatment total)/healthy total.

UDC, untreated disease control; TM, tiophanate-methyl WP fungicide.

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**Fig. 2.** Inhibition of the fungal fruiting body and rooting induction after drench treatment of 62.5 ml/l NaOCl to grafting region diseased by *Helicobasidium mompa* on July-August. (A) The fungal fruiting body occurred to grafting region pre-treatment (September 17, 2014). (B) Root primordia (arrow) induced post-treatment (July 8, 2013). (C) Rooting (arrow) induced post-treatment (August 6, 2014).
The number of shoots by treatment frequency. The number of shoot lengths by treatment frequency with NaOCl 31.25 and 62.5 ml/l is shown in Fig. 4. According to the survey of the number of shoots surveyed before treatments, the number of short shoots (5 cm or less) was ranged from 8 to 9.7 without significance among treatments, that of intermediate shoots (5 to 30 cm) was ranged from 1.3 to 4.3 without significance, that of long shoots (30 cm or more) was absent in all treatments, and the total number of shoots was ranged from 9.3 to 14 without significance among treatments. In the survey after once treatment, the number of short shoots was varied from 0 to 1.7 without significance among treatments, and that of intermediate shoots was ranged from 8.3 to 18.3, as the treatment with NaOCl 62.5 ml/l only showed a significant difference without significance between that of NaOCl 31.25 and 62.5 ml/l, the number of long ones was ranged from 0.3 to 3.3 without significance among treatments. The total number of shoots was varied from 10 to 21.7 with significance in the treatment of NaOCl 62.5 ml/l. In the survey after two-time treatments, the number of short shoots for treatments

Fig. 3. The relative tree crown density by treatment frequency for apple trees infected by Helicobasidium mompa in an apple farm. Surveyed date were August 6, 2014, August 4, 2015, and August 8, 2016. Mean ± standard deviation followed by different letter over each bar indicates significant difference based on Duncan’s multiple range test at \( P < 0.05 \). UDC, untreated disease control; TM, tiophanate-methyl WP fungicide.

Fig. 4. Change in the number of shoots by length by treatment frequency with the available chlorine content of NaOCl on apple trees infected by Helicobasidium mompa in an apple farm. Treatment frequency with available chlorine content of NaOCl were as follows; pre-treatment (A), after once treatment (B), twice (C), and thrice (D). The surveyed date of pre-treatment was May 26, 2014, the survey for once treatment was on August 6, 2014. The survey for two-time treatments was conducted on October 13, 2015. The survey for three-time treatments was conducted on October 25, 2016. Mean ± standard deviation followed by different letter over each bar indicates significant difference based on Duncan’s multiple range test at \( P < 0.05 \). UDC, untreated disease control; TM, tiophanate-methyl WP fungicide.
of NaOCl 31.25 and 62.5 ml/l was from 36.3 to 43.7 with significance, respectively, as the intermediate shoots for the treatments of NaOCl 31.25 and 62.5 ml/l was ranged between 27.3 and 48.7 with significance, respectively. The treatment of NaOCl 62.5 ml/l was more significantly effective than that of NaOCl 31.25 ml/l. The long shoots was ranged from 0 to 2.3 with significance in NaOCl 62.5 ml/l. The total number of shoots with treatments of NaOCl 31.25 and 62.5 ml/l were 64.3 and 94.7 with significance, respectively. The treatment of NaOCl 62.5 ml/l was more significantly effective than that of NaOCl 31.25 ml/l. In the survey after three-time treatments, despite of the dieback both the UDC and TM plot, the number of short shoots with treatments of NaOCl 31.25 and 62.5 ml/l were 34.7 and 41.7 with significance, respectively. The intermediate shoots for treatments of NaOCl 31.25 and 62.5 ml/l were 39.7 and 57 with significance, respectively. The treatment of NaOCl 62.5 ml/l was more significantly effective than that of NaOCl 31.25 ml/l. The long shoots with NaOCl 62.5 ml/l were 6.3, which showed only a significant difference with the treatment. The total number of shoots for treatments of NaOCl 31.25 and 62.5 ml/l were 78 and 105 with significance, respectively. Additionally, the treatment of NaOCl 62.5 ml/l was more significantly effective than that of NaOCl 31.25 ml/l.

In this study, two- or three-time treatments of NaOCl 31.25 and 62.5 ml/l from late fall to early spring reduced dieback as well as improved the tree crown vigor through extinction of target pathogen for apple trees affected by violet root rot. Recently Lee et al. (2019) reported that *H. mompa* could be entirely inhibited through contact with 31.25 and 62.5 ml/l available chlorine content of NaOCl over each 160 and 320 s. As Shin et al. (2014) reported that NaOCl had good permeability, we assumed that it was able to control violet root rot effectively with permeability of NaOCl, promote the rooting on rootstock, and supply nutrient and water to vascular tissue in the infected trees.

On the other hand, the TM plot showed an insignificant control effect to violet root rot. *H. mompa* is likely to be acquired resistance or cross-resistance for TM, because of the use of fungicides at least 10 times per year. Fungicide Resistance Action Committee (2017) reported that thiophanate-methyl showed resistance in many pathogenic fungi and cross-resistance within a carbamate group.

The number of fruit-setting, fruit characteristics, and total starch content of fruit-bearing shoots by treatment frequency. The number of fruit-setting by treatment frequency with NaOCl 31.25 and 62.5 ml/l is shown in Fig. 5. With once treatment in 2014, there was a significant difference only for the treatment of NaOCl 31.25 ml/l and no significance between that of NaOCl, compared to the UDC plot. The number of fruit-setting of each treatment were ranged from 21 to 31 (that of healthy trees were 57). With two-time treatments in 2015, there was a significant difference for both NaOCl 31.25 and 62.5 ml/l and the number of fruit-setting for each treatment that were varied from 16.7 to 43.7 (that of healthy trees were 70.7). With three-time treatments in 2015-2016, both NaOCl 31.25 and 62.5 ml/l treatment showed a significant difference. The number of fruit-setting for the other treatments except for the TM plot treatment were ranged from 13 to 52.7 (that of healthy trees were 81.7). We found that two or more drenching treatments of NaOCl 31.25 and 62.5 ml/l restored the tree vigor without dieback of apple trees to about 57-65%, compared to the number of fruit-setting on healthy trees.

Fruit characteristics of the infected trees by treatment frequency with NaOCl 31.25 and 62.5 ml/l are shown in Table 3. About once treatment in 2014, there was a significant difference for both NaOCl treatments without difference between two concentrations of NaOCl for fruit weight, length, and total soluble solid, compared to those of the UDC. About both two-time treatments in 2015 and three-time treatments in 2015-2016, there was a significant difference for both NaOCl treatments without any difference between two concentrations of NaOCl for fruit weight, diameter, length, and firmness. The TM plot showed no data because of dieback overall in the treatment plot. On the other hand, the two-way ANOVA results showed the significant differences among treatments, treatment...
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frequency, and interaction between treatments and treatment frequency. Therefore, it is indicated that two or more drenching treatments of NaOCl 31.25 and 62.5 ml/l from late fall to early spring would improve marketability of the fruits like healthy trees and help trees regain vigor.

The total starch content of fruit-bearing shoots for the infected trees by treatment frequency with NaOCl 31.25 and 62.5 ml/l is shown in Fig. 6. With once treatment in 2014, there was a significant difference for all treatments, compared to the UDC. In particular, both NaOCl treatments showed more increased total starch contents than that of the healthy trees. After the treatments in 2015 as well as in 2015-2016, there were significant differences for both NaOCl treatments and the total starch content for both NaOCl treatments was almost the same as that of the healthy trees. Therefore, it is indicated that two or more drenching treatments of NaOCl 31.25 and 62.5 ml/l from late fall to early spring would increase the total starch content of fruit-bearing shoots in the infected trees, suggesting tree vigor recovery through an increment in stored nutrients.

Table 3. The fruit characteristics by treatment frequency on apple trees infected by Helicobasidium mompa in an apple farm

| Treatment (A) | Frequency (B) | Fruit weight (g) | Fruit diameter (mm) | Fruit length (mm) | Firmness (N) | Total soluble solid (°Brix) | Titratable acidity (%) |
|---------------|---------------|------------------|---------------------|-------------------|--------------|----------------------------|-----------------------|
| UDC           | Once          | 189 c            | 77.1 b              | 64.3 e            | 95.0 a       | 13.2 c                     | 0.47 a                |
|               | Twice         | 160 b            | 71.2 b              | 66.6 b            | 66.7 b       | 14.0 a                     | 0.40 b                |
|               | Thrice        | 156 b            | 70.9 b              | 66.1 b            | 64.8 b       | 14.2 a                     | 0.39 b                |
| TM            | Once          | 201 bc           | 78.0 b              | 65.4 bc           | 96.6 a       | 13.1 bc                    | 0.45 a                |
|               | Twice         | 166 b            | 72.1 b              | 68.3 b            | 66.7 b       | 13.7 ab                    | 0.40 b                |
|               | Thrice        | - c              | - c                 | - c               | - c          | - c                        | - c                   |
| 31.25 ml/l NaOCl | Once        | 217 ab           | 80.5 a              | 66.6 ab           | 97.8 a       | 14.0 a                     | 0.46 a                |
|               | Twice         | 228 a            | 79.8 a              | 75.1 a            | 91.1 a       | 13.8 ab                    | 0.42 b                |
|               | Thrice        | 224 a            | 79.0a               | 74.2 a            | 89.6 a       | 13.9 a                     | 0.41 ab               |
| 62.5 ml/l NaOCl | Once        | 210 a            | 78.8 b              | 67.9 a            | 94.9 a       | 13.8 ab                    | 0.46 a                |
|               | Twice         | 231 a            | 81.4 a              | 76.4 a            | 90.7 a       | 13.5 b                     | 0.43 a                |
|               | Thrice        | 229 a            | 80.6 a              | 75.7 a            | 87.8 a       | 13.6 b                     | 0.43 a                |

Two-way ANOVA

A***

B***

A×B***

Mean followed by different letters within columns indicates significant difference based on Duncan’s multiple range test at $P < 0.05$. ***Significant at $P < 0.001$.

UDC, untreated disease control; TM, tiophanate-methyl WP fungicide.

Fig. 6. The total starch content of fruit-bearing shoots by treatment frequency on apple trees infected by Helicobasidium mompa in an apple farm. Mean ± standard deviation followed by different letter over each bar indicates significant differences based on Duncan’s multiple range test at $P < 0.05$. UDC, untreated disease control; TM, tiophanate-methyl WP fungicide.
tree vigor up to 60% level of healthy trees with an increase in fruit-setting, improvement of fruit quality, and increment of total starch content in fruit-bearing twigs. Similar to our results, Bisessar and McIlveen (1992) reported that treatments of hypochlorous acid 1.5 and 150 ml/l increased significantly the length of shoots, fresh weight, and dry weight in *Poa pratensis*.

In increasing the control effect of NaOCl on the disease, the soil texture of damaged apple orchards and the timing of its application are important factors. This is because they have a significant effect on the contact time between it and the pathogen (Cayanan et al., 2009; Copes et al., 2001; Datnoff et al., 1987; Jeffers, 1992; Johnson et al., 1997; Lee et al., 2019; Santos-Rufo and Rodriguez-Jurado, 2016; Segall, 1968). As for the soil texture, in areas with poor vertical drainage, the diluted solution of it does not permeate properly and remains stagnant in the soil, which decreases the possibility of the its contact with pathogens. Thus, loam soil or sandy loam soil with smooth vertical drainage that allows the diluted solution of it to permeate in the rhizosphere as quickly as possible is suitable. Meanwhile, the timing of its application is closely related to the temperature. It has a high vapor pressure of 17.4-20 hPa at 20°C (http://ecb.jrc.it/esis; https://msds.kosha.or.kr/), which means only when conditions of low soil and water temperature are met, the possibility of contact between it and pathogens can be increased and the effect of disease control could be maximized. So, the its application is effective between autumn and early spring when the temperature of soil and water in which it is diluted is low, but in summer when the temperature is high, it has little effect (no data).

Therefore, our findings from this study strongly indicates that the drenching application of 31.25-62.5 ml/l available chlorine contents in NaOCl from late fall to early spring to apple trees infected by *H. mompa* increases the efficacy of both disease control and potential recovery of tree vigor. Furthermore, we propose a standard method for apple farms with other soil-borne disease problems.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

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