Outbreaks of Yuzu Dieback in Goheung Area: Possible Causes Deduced from Weather Extremes

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Starting in 2012, severe diebacks usually accompanied by abundant gum exudation have occurred on yuzu trees in Goheung-gun, Jeonnam Province, where severely affected trees were occasionally killed. On-farm surveys were conducted at 30 randomly-selected orchards located at Pungyang-myeon, Goheung-gun, and the resulting disease incidences were 18.5% and 39.6% for dieback and gumming symptoms, respectively. Black spots on branches and leaves also appeared on infected trees showing a typical dieback symptom. Morphological and molecular identifications of the isolated fungal organisms from lesions on the symptomatic leaves and branches revealed that they are identical to Phomopsis citri, known to cause gummosis. In order to find the reason for this sudden epidemic, we investigated the weather conditions that are exclusively distinct from previous years, hypothesizing that certain weather extremes might have caused the severe induction of pre-existing disease for yuzu. There were two extreme temperature drops beyond the yuzu’s cold hardiness limit right after an abnormally-warm-temperature-rise during the winter of 2011–12, which could cause severe frost damage resulting in mechanical injuries and physiological weakness to the affected trees. Furthermore, there was an increased frequency of strong wind events, seven times in 2012 compared to only a few times in the previous years, that could also lead to extensive injuries on branches. In conclusion, we estimated that the possible damages by severe frost and frequent strong wind events during 2012 could cause the yuzu trees to be vulnerable to subsequent fungal infection by providing physical entries and increasing plant susceptibility to infections.

Keywords: dieback, gummosis, Phomopsis, yuzu

Yuzu is a perennial woody evergreen tree, famous for its aromatic fruits, that grows not only in Korea but also in China and Japan. Among them, the Korean yuzu is notable for its fragrance. Since 2000, demand for yuzus has increased due to a growing interest in health and well-being in Korea. In a very short time yuzu production has increased greatly, reaching a cultivation area of 2,237 ha and 19,127 metric tons of annual products, while its market price has soared since 2005. Pungyang-myeon, Goheung-gun in Jeonnam Province is the most intensive cultivating area for yuzu in Korea. This study was initiated in 2013 because almost one third of yuzu trees in Pungyang-myeon were severely damaged during a sudden dieback caused by unknown reason. Based on some of the characteristic symptoms, disease infection caused by certain pathogens was pointed to as the probable main cause, with additional damages from environmental conditions such as extreme frost and strong winds from typhoons also playing a role. Among the major diseases affecting yuzu tree are melanose, gummosis, stem pitting, anthracnose, Phytophthora blight, and others (KSPP, 2009). In particular, melanose and gummosis affect almost all parts of the tree, such as the leaves, branches, and the fruit, causing significant decreases in both the quantity and quality of the fruits (Hur and Park, 2005a; Hur and Park, 2005b; KSPP, 2009; Ryu et al., 1993). It is also known that the gummosis fungus generally penetrates trees through external wounds such as scars on branches and trunks, branch-diverging sites, injury sites from pruning, frost, and sunburn (Akira, 1981). While some cases of citrus gummosis were previously reported in Korea (Hyun et al., 2002; KSPP, 2009), few studies have been conducted on yuzu gummosis. Nevertheless, the occurrence of gummosis on yuzu has been long perceived in Korea because similar symptoms have been reported.

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in previous studies (Kim et al., 1997; Koh et al., 1996; Kong, 1994;). Jee et al. (1997) reported an occurrence of yuzu gummosis caused by Phytophthora nicotianae in the southern parts of South Korea. Since the causing fungus is known as a soil-born pathogen, the symptoms started at the grafting sites below the soil line, mainly affecting lower trunks of yuzu trees. It was noted that the symptoms reported by Jee et al. (1997) were largely different from the ones surveyed in this study, thus we hypothesized that the gummosis in Goheung-gun might be caused by another agent. Therefore, the purpose of this study was to identify the root cause of the yuzu gummosis and dieback that happened in Goheung-gun during 2012–13, which can potentially be utilized to develop farm management protocols for yuzu growers to cope with similar risks in the future. In this study, we successfully isolated and identified the causal agent of the yuzu gummosis and dieback. Additionally, the weather conditions of Goheung-gun were thoroughly analyzed to find any relationship with the disease outbreak. As a result, we found out that the weather conditions seemed

Fig. 1. Symptoms of gummosis on the yuzu trees. (A) Gummosis infected orchard showing severe diebacks. (B) Cuttings of the severely affected yuzu branches. (C) Outer symptom of gumming on a trunk. (D) Inner symptom of gumming on a trunk. (E) Black spots on twigs. (F) Black spots on leaves. (G) Gumming fruit.
to be highly correlated with the yuzu gummosis and dieback that happened in Goheung-gun, Jeonnam Province.

As the first step in the study, we visited the yuzu orchards located at Pungyang-myeon, Goheung-gun, and investigated the actual incidence of the dieback with gumming symptoms on branches and trunks of yuzu trees. Major symptoms were investigated as shown in Fig. 1. There were frequent gumming symptoms observed on branches and trunks, where tree bark appeared oil-soaked and tan resin or gum exudate came off the trees like heavy bacterial ooze (Fig. 1C). Fruits on severely infected trees showed the gumming symptom as well (Fig. 1G). When peeling off the symptomatic parts with a knife, the rotten epidermis and discolored xylem parts appeared. The boundary between the infected and healthy parts was clearly distinguishable due to vivid color differences (Fig. 1D). Another frequent symptom was black spots on twigs and leaves as shown in Fig. 1E and F, similar to a typical melanose symptom on a citrus tree. In many of the infected trees, the branches and trunks at the top of the infected site withered and died (Fig. 1A and B). It seemed that the infection expanded deep into the vascular bundle of the affected trees and the necrosis of the vascular part caused the complete blockage of the water supply through the xylem, eventually resulting in the severe dieback symptom.

Rates of incidence for these two distinct symptoms, represented as dieback and gumming on branches and trunks, were quantitatively measured. Yuzu orchards located at Pungyang-myeon, Goheung-gun were subject to the investigation. From June to July in 2013, we visited 30 orchards located in five villages of Pungyang-myeon to investigate the disease incidence. Out of the total 2,886 yuzu trees surveyed, 818 yuzu trees were randomly selected for the investigation. As previously mentioned, incidences for dieback and gumming symptoms were separately measured. Symptoms of dieback were also found on small branches or twigs of yuzu tree’s upper end. Thus, we calculated the incidence of dieback at a rate of symptomatic branches out of the total investigated branches. For the gumming symptom, which is also a typical symptom of gummosis, the incidence of gumming was calculated at a rate of symptomatic trunks out of the total investigated trunks since it was normally seen in the main branches or trunks.

Table 1 shows the orchard investigation results for the incidences of two major symptoms. Mean incidences of the dieback and gumming symptoms were 17.5% and 42.7%, respectively. It was noted that there were large differences in the incidence rates depending on the surveyed orchards (data not shown). For example, the dieback incidence differed to a maximum of five times, from the lowest (7.3%) to the highest (36.5%), while the gumming incidence ranged from 12.0% to 74.8%, almost six times of the difference between orchards.

Generally, gummosis in citrus refers to any gumming symptoms, which may be a direct or indirect result from infections by plant pathogens, physical damages by insect or environmental factors. However, it was later reported that many of the gumming symptoms on citrus were actually related to the gummosis fungus, *Phomopsis citri* (Ryuji et al., 1992; KSPP, 2009). A fungus belonging to the *Phomopsis* genus was described as a possible causal agent for the yuzu gummosis in the previous studies, yet its full species name has not been investigated so far (Kong, 1994; Kim et al., 1997). To fill in this missing information, we isolated and identified potentially-pathogenic microorganisms in the diseased parts of a yuzu tree such as the leaves, branches and stems. Briefly, plant sections of twigs, branches, and trunks showing the symptoms of gummosis were cut in 0.5 × 0.5 cm, and washed with 70% ethanol for 30 seconds, followed by a washing in sterile water for one minute. After absorbing moisture with a sterile filter paper, the plant sections were inoculated on potato dextrose agar (PDA) medium, subsequently incubated for three days at 25°C, and each resulting hyphal agar block was transferred to a new PDA medium. There was one major fungal colony isolated in the study. Mycological features such as

| Locations  | Number of orchards | Number of surveyed trees | Incidence rate (%) |
|------------|---------------------|--------------------------|-------------------|
|            |                     |                          | dieback | gumming     |
| Daecheong  | 6                   | 210                      | 20.5    | 43.0        |
| Handong    | 4                   | 100                      | 15.9    | 50.5        |
| Hanseo     | 4                   | 120                      | 11.5    | 41.6        |
| Bongyang   | 10                  | 239                      | 21.1    | 49.4        |
| Singi      | 6                   | 149                      | 13.4    | 26.7        |
| **Total**  | **30**              | **818**                  | **17.5** | **42.7**    |
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Color, shape and size of the mycelial colony and conidia collected from the colony on the medium were investigated through a microscope (Fig. 2A and B). Two distinguished conidia were retrieved. Microscopic phenotypes of the isolated conidia were compared with the previously-reported P. citri conidia as a reference (Table 2). As shown in Fig. 2C and D, α-conidia was shaped spindle or oval with the size of 5.7–9.6 × 2.1–3.8 μm, and colorless and transparent β-conidia looked like a thin wire and had a hook-shaped curve with a size of 20.8–30 × 0.6–1.4 μm. We concluded that these conidial features exactly matched with the P. citri that Fawcett (1912) and Punithalingam and Holliday (1973) had reported in their previous studies.

Melanose (or black spot disease) is one of the most economically important diseases in citrus and yuzu in most citrus-growing areas in the world, and the disease is caused by Diaporthe citri F.A. Wolf (anamorph: Phomopsis citri H. Fawc.) (Whiteside, 2000). The anamorphic stage (P. citri) of the melanose pathogen was reported to be responsible for gummossis on citrus in Korea and Japan (Akira, 1981; Hyun et al., 2002; Koh et al., 1996; KSPP, 2009; Ryuji et al., 1992). In this study, we revealed that the gummossis of yuzu is also caused by P. citri, the same fungus that causes gummossis in citrus. Nevertheless, the symptoms and etiology of melanose and gummossis are different from each other. For example, the melanose fungus forms pycnidia and perithecia on dead twigs, and does not thrive in a living tissue unlike gummossis (Whiteside, 2000). Therefore, we hypothesized that the same fungus may be able to cause both melanose and gummossis in yuzu. In other words, the physiological state of the host possibly affects the consequence of infection by the same fungal pathogen, either melanose or gummossis. Since the P. citri is not known as a highly virulent pathogen, it is generally not capable of causing severe infection to a healthy host. In this case, it will remain as a moderate or somewhat facultative pathogen, resulting in melanose symptoms on dead twigs. However, when the host suffers from external damages like frost, sunburn, and strong wind, or physiological stresses from excessive pruning, excessive use of fertilizer, and

Fig. 2. Morphological characteristics of Phomopsis citri, the causal agent of yuzu gummossis. (A) A fungal colony grown for 6 days on a potato dextrose agar medium. (B) A fungal colony grown for 60 days on a potato dextrose agar medium. (C) α-conidia of P. citri. (D) β-conidia of P. citri.
excess fruiting, the fungus may have a strong chance to cause severe infection of the host (Hyun et al., 2002). This is probable due to the defense mechanisms of host plants weakening or deteriorating due to damages and excessive stress (Akira, 1981; Freeman and Beattie, 2008), which normally allows for a fast track of penetration and colonization of the pathogen.

In this regard, we hypothesized that the outbreak of yuzu gummosis in Goheung-gun might be resulting from an exposure of yuzu trees to unusual environmental stresses. To investigate this hypothesis, we analyzed daily weather conditions during the period of 2011–12. Extreme anomalies from a climatological normal (past 30 years) were a major target for this investigation. Through this analysis we might be able to find the hidden relationship between the gummosis outbreak and historical weather conditions in Goheung-gun.

Growing yuzu requires an annual average temperature of 14–15°C and a daily temperature range of 15–20°C. It is said that cultivating yuzu at an inland area is difficult because a sea breeze is necessary for its growth. This may explain why most yuzu trees are grown in the southern coastal areas of Jeonnam and Gyeonnam Provinces and in the outer parts of Jeju Island in South Korea. Yuzu possesses the strongest cold resistance among citrus, thus it can grow in locations where winter temperature can drop down to −9°C (Ikeda et al., 1980). Some literature indicate that yuzu is subject to possible frost damage at −10°C when it is at a mature stage, and −8°C at its juvenile stage (Kong, 1994; Kim et al., 1997). Although it has been stated that yuzu can survive even at −12°C, we believe that it may physiologically undergo considerable damage below −10°C based on a previous study reporting that some yuzu trees died after

| Characteristics | Isolated conidia in this study | Phomopsis citri* |
|-----------------|--------------------------------|-----------------|
| **α-conidia**   |                                |                 |
| Size (µm)       | 5.7–9.6 × 2.1–3.8              | 6.0–10 × 2.0–3.0|
| **β-conidia**   |                                |                 |
| Size (µm)       | 20.8–30 × 0.6–1.4              | 20–30 × 0.5–1.0 |

*aData from Punithalingam and Holliday (1973)

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Table 2. Comparison of the conidial characteristics of isolated conidia in the study with the reported ones of Phomopsis citri

Fig. 3. Annual variations of the monthly minimum value of daily minimum temperatures in Goheung for December, January, February, and March from 2000 to 2013. Red dotted lines in each graph indicate a climatological value of the monthly minimum of daily minimum temperatures during 1981–2010 as a reference for comparison.
temperatures fell to $-10.28^\circ C$ (Rieger et al., 2003).

During winter, yuzu trees go through the cold acclimation process as the temperature decreases slowly. Once dormancy starts after the cold acclimation, a tree becomes hardy enough to endure extreme cold temperature (Cooper, 1965; Irving and Lanphear, 1967). However, once February starts and temperatures begin to increase, the dormancy of the tree breaks and the physiological metabolism re-starts rapidly. This is the period when yuzu loses its cold hardiness and become significantly vulnerable to frost damage, especially when temperatures drop sharply. To find out more about this issue, we analyzed the temporal variation of daily minimum temperature, especially during winter seasons. Daily weather data were obtained from the Korea Meteorological Administration (KMA) automatic synoptic observation system (ASOS) station in Goheung-gun (ID262). Graphs in Fig. 3 show the variations of the monthly minimum value of daily minimum temperatures from 2000 to 2013 for the month of December, January, February, and March. Red dotted lines indicate the climatological value of the monthly minimum of daily minimum temperatures during 1981–2010. There were five years (2000, 2003, 2009, 2012, and 2013) when the minimum temperature in January fell below $-10^\circ C$, the hardness limit of yuzu in a deep dormancy. Although it depends on many other factors such as variety, plant age, nutritional state, and anti-freezing protection, falling under $-10^\circ C$ may have caused some physiological shocks to yuzu in those years. In February 2012, the minimum temperature dropped all the way down to $-11^\circ C$, which was a huge anomaly compared to the climatological normal, $-8.18^\circ C$. Considering that the outbreak of yuzu gummosis started in the middle of 2012, this abnormal minimum temperature in February might have played a role in the disease outbreak.

To investigate a potential impact of the abnormal minimum temperature on the physiology of yuzu tree, we looked at daily variations of the minimum temperature from December 2011 to February 2012 in Fig. 4. Notably there were days when the minimum temperature increased to 6.7°C in the middle of January. This abnormally-warm period was quite enough to trigger the deacclimation process, breaking dormancy in the middle of winter. More seriously, the temperature suddenly dropped down to below $-10^\circ C$ within six days after the abnormal rise to 6.7°C. This sharp temperature drop should cause severe frost damage to yuzu trees with a high possibility. In contrast to the winter season of 2011–12, the daily variations in 2008–09 showed a typical climate condition that is comparable to a

![Fig. 4. Daily variations of the minimum temperature during winter season (December to February) in Goheung. Red circles on each graph indicate the highest or lowest temperatures during the specific season. (A) December, 2011 to February, 2012 (B) December, 2008 to February, 2009.](image-url)
climatological normal winter. Comparing this graph with the one of 2011–2012, we were certain that the winter season of 2011–2012 was an extraordinary environment for yuzu trees in Goheung-gun.

To experimentally confirm the possible damage of yuzu trees by low temperature, we grew yuzu seedlings in a cold chamber at different temperatures. Our results showed that even at −5°C, typical frost damage symptoms such as cracks on branches and twigs were observed within three days of incubation (data not shown). Incubating yuzu seedlings at −9°C caused severe leaf wilting and dieback of branches within 1–2 hours. Leaf wilting became more severe with moisture in the leaves. Since we used seedlings instead of mature trees, the responses might be more exaggerated than a natural field situation. In addition, we had to treat the seedlings with a gradual temperature drop for an acclimation process during winter, which should provide more cold resistance for the seedlings. However, it was enough to predict from these observations that possible physiological deterioration as well as physical cracks from the frost damage might have a significant impact on yuzu gummosis occurrence.

The south coastal area of Korea is often affected by monsoon winds and typhoons from spring to late summer. The impacts from typhoons especially could result in severe physical damage to yuzu trees in this area (Kong, 1994; Kim et al., 1997). Although physical damage itself may not be fatal to the tree, scars from heavy defoliation and scratches by thorns on a branch or bumping between branches could play a significant role of providing natural openings for pathogenic penetration (Tainter, 1996). There were four major typhoons that affected Goheung in 2012, starting from Kanun with a maximum wind speed of 25 m/s, Bolaben with 65 m/s, Denbin with 60 m/s, and a super typhoon Sambar with a maximum wind speed of 75 m/s. It was very unusual in terms of the typhoon frequency compared to other years.

To estimate the relationship between yuzu gummosis epidemic and the typhoons that occurred in 2012, we checked the daily maximum instantaneous wind speed in Goheung. First, we decided a threshold for the maximum instantaneous wind speed which is enough to inflict mechanical damages to yuzu. A wind speed of 20 m/s was determined as a threshold based on the damage criterion in the Crop Disaster Insurance for Wind and Flooding by the NongHyup Property & Casualty Insurance Co., Ltd. in Korea. We calculated the frequency of daily maximum instantaneous wind speed exceeding the threshold. As shown in Fig. 5, a repeated pattern of spring monsoon winds were observed every year, except from 2001 to 2003, which exhibited weak patterns. Sharp increases in wind speed appeared occasionally in the summer, probably due to typhoons. The frequency of maximum instantaneous wind speed over 20 m/s showed an irregular pattern of 0–3 times every year. However, it was noted that the frequency unprecedentedly increased to seven times in 2012. This was consistent with the fact that there were more occurrences of typhoons in that year compared to other climatological normal years.

It can be inferred that there was a large amount of physical damage to yuzu trees from this unprecedented occurrence of strong winds in 2012. Local growers also stated that due to the severe defoliation of leaves, yuzu fruits were exposed to strong sunlight in the summer and autumn, resulting in sunburns on fruits. Considering that scars on a plant become an easy entry point or a penetration site for plant pathogens, it is rational to expect more infections on scarred plants than unaffected ones as long as there is an enough inoculum available. Physical damage also leads to physiological stress on plants. Under severe stress, the defense system of a host plant may not be able to play a role in a proper way. This is another reason that more infec-

![Fig. 5. Daily variation of maximum instantaneous wind speed and annual frequency of strong winds in Goheung. Red bars on the graph highlight strong winds exceeding the threshold for strong wind: 20 m/s.](image-url)
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In this study, we tried to find rational and logical reasons for the yuzu gummosis outbreak in Goheung-gun in 2012 by investigating pathogenic agents directly causing the disease and other impacts from abnormal weather conditions that could make the yuzu vulnerable to the infection. In a normal situation, the gummosis fungus would not be able to cause a severe disease outbreak on yuzu because of its relatively weak virulence. However, the chances of infection become higher when the plants become susceptible due to environmental causes like the incidences that occurred in Goheung-gun. We conclude that the frost damage from sudden temperature drop following abnormal temperature rise in the middle of January did sufficiently make the yuzu susceptible to the disease. In addition, unprecedented strong winds over 20 m/s in 2012 were also assumed to be an additional environmental cause to drive the yuzu to a susceptible state, resulting in severe onset of gummosis disease.

In order to prevent another gummosis or other disease outbreaks, there needs to be a greater effort to protect yuzu trees from unexpected extreme climate conditions. Disease outbreaks can be minimized by maintaining good fertilization and cultural practices and proper controlling practices for pests and diseases. As climate change continues, more frequent and intensified climate variability should be expected. More climate variability generally refers to more extreme environmental conditions, which will surely make plants more susceptible to diseases and pests in the future. To make matters worse, it appears that it is not easy to equip an individual grower with the ability to cope with these problems. Therefore, it is urgent that the growers are prepared by all possible means from scientific development and policy-oriented intervention.

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