Effects of the Contents of Mineral Elements on Gummosis in *Prunus salicina* Lindl.

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Abstract. To investigate the relationship between mineral elements and plum gummosis disease, *Prunus salicina* Lindl. trees with four grades of gummosis were used as the experimental materials. The contents of N, P, K, Ca, Mg, B, Fe, Mn, Zn and Cu in the branches and leaves were measured, and the correlation between mineral elements and gummosis was systemically analyzed through multiple comparisons, binary logistic regression analysis, and ordinal logistic regression analysis. In addition, the effects of prevention and control of the necessary mineral elements on the gummosis disease of *P. salicina* were verified after a fertilization experiment. The results indicate that the contents of nitrogen and manganese positively correlate with the occurrence of gummosis. In contrast, the contents of calcium and boron significantly negatively correlate with the occurrence of gummosis. A fertilization experiment facilitated the discovery that the control of nitrogen, as well as the increased application of boron and calcium fertilizers, contributed to the prevention and control on gummosis disease in *P. salicina*

Prunus salicina Lindl. is native to Yunan and distributed in southwestern provinces in China where it has been cultivated for many years. Gummosis is a global disease, which occurs mainly on the tree trunk, main branch, and the collateral. At the early stage of the disease, the lenticels flow out of the light yellow transparent colloid which gradually becomes reddish brown after condensation, and the cortex and xylem of the site of the disease bulge and gradually decay and brown. When the disease is serious, it is difficult to control, and weak trees will die (Anil et al., 2017; Li et al., 2019; Younguk et al., 2008).

Many factors cause gummosis, for example mechanical damage, planting environment, and pathogenic bacteria. Therefore, current prevention and control of the disease mainly focus on the selection of disease-resistant varieties, rational fertilization and pruning, and other agricultural control, supplemented by chemical control.

Previous research found that the ingredients of flowing colloids from stone fruit trees are primarily polysaccharides. Some researchers hypothesized that this colloid is composed of complex multibranched cell wall polysaccharides (Boothby, 1983; Simas et al., 2008; Simas-Tosin et al., 2010). Both

boron and calcium are involved in the composition of cell walls and can maintain their structure and function, while the mineral elements including nitrogen and potassium play roles in the physiological activities of fruits. Many experts conducted research on fruit cracking of pomegranate (Bakeer, 2016), sunburn of apple (Lötze et al., 2018), top rot in persimmon (Tang et al., 2013), jujube fruit-shrink disease (Zhang et al., 2019), and apple bitter pit (Wallace and Jones, 2015). They found that there is close correlation between these physiological diseases and mineral nutrition. However, current research on gummosis emphasizes the isolation and identification of infectious pathogens and the selection of fungicides. Most of them focus on the physiology and control of gummosis in peach and cherry (Beckman et al., 2003; Benfradj et al., 2017; Wang et al., 2011). There has been relatively little research on mineral nutrition, particularly the effects of lack of mineral elements on gummosis in plum trees. Therefore, in this study, *P. salicina*, which is prone to gummosis, was used as the test material. The different contents of mineral elements in the branches and leaves of *P. salicina* without gummosis and trees with different grades of gummosis were analyzed. In addition, binary logistic regression analysis and ordinal logistics regression analysis through SPSS 18.0 were respectively applied to study the correlation between mineral elements and existence of the gummosis and correlation between mineral elements and occurrence grades of gummosis. The ability of the external addition of minerals needed to prevent and control the gummosis disease of *P. salicina* was verified. This experiment aimed to illustrate the mineral nutrition physiology of the tree, as well as the

ability of spraying fertilizers to prevent and control gummosis on *P. salicina*.

Materials and Methods

Experimental site. The experimental site is at Shilian Village, Fenshui Town, Wanzhou District, Chongqing City, China, located at a subtropical humid monsoon climate zone. It is featured with four distinctive seasons, abundant sunshine and rainfall, as well as long frost-free period. The annual average temperature is 17.7°C, the mean annual sunshine duration is 1484.4 h, and the mean annual precipitation is 1243 mm. The experimental fields comprise flat, red sandy soil. The basic physicochemical properties are as follows: pH 5.83, organic matter 22.80 g/kg, available nitrogen 93.88 mg/kg, available phosphorus 23.75 mg/kg, available potassium 99.51 mg/kg, exchangeable calcium 1270.0 mg/kg, exchangeable magnesium 205.0 mg/kg, available zinc 1.61 mg/kg, available iron 7.77 mg/kg, available manganese 8.37 mg/kg, available copper 0.81 mg/kg, and available boron 0.58 mg/kg.

Plant materials and degrees of gummosis. To date, there was no standard used to rate the severity of this disease. Therefore, we graded the different degrees of gummosis in *P. salicina* (Table 1) based on the grading standard of peach gummosis disease used by Luo et al. (2005).

Sampling. In a high incidence season of *P. salicina* gummosis, 100 plums were selected from 20 green plum trees of each degree of gummosis. Four vegetative branches from the direction of east, west, north, and south of each tree, as well as all the leaves on the selected branches, were collected. The branches and leaves were taken back to the laboratory immediately after collection. No fertilizers were applied during the sampling period.

Determination of mineral elements. According to the standard established by China Agricultural University (Beijing, China), the samples were cleaned as follows: 1) 0.1 mol/L hydrochloric acid was used to wash the leaves for 30 s; 2) 0.1% White Cat cleaning agent (Shanghai He Yellow and White Cat Co. Ltd) was used for 30 s; 3) samples were removed from the solution and washed with tap water; and 4) deionized water was used for washing. Filter paper was used to absorb the surface moisture. The washed leaves were dried for 20 min in an oven at 105°C to denature the enzymes and then dried at 70 to 80°C for 48 h. The leaves were grinded using a stainless-steel plant grinder, screened through a 0.25-mm mesh sieve, and stored in a Hebi Rip dryer (Shenzhen Gangyi Screen Printing Machinery CO., Ltd., GY-HB120150) for determination. The amounts of N and P were measured using H₂SO₄-H₂O₂ digestion. Total nitrogen was determined using the Kjeldahl method (Hangzhou Top Instrument Co., Ltd., ZDDN-II). Total phosphorus was determined using molybdenum-antimony-scandium colorimetry (Bao, 2000). The amounts of K, Ca, Mg, Fe, Mn, Zn, and Cu were determined using a dry-ashing method and measured by using an

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Table 1. The grading standard of peach gummosis disease.

Grade	Grading standard ²
0	The whole plant is normal with no gummosis or lesions
1	1–9 gummosis points on the tree. The area of disease comprised <5% of the trunk area.
2	10–19 gummosis points on tree. The area of disease comprised ≈5% to 10% of the trunk area.
3	20–29 gummosis points on the tree. The area of disease comprised ≈10% to 20% of the trunk area.
4	>30 gummosis points on the tree. The area of disease comprised >20% of the trunk area.

²When the gummosis points and disease area conform to different degrees of disease, the higher degree was recorded.

atomic absorption spectrophotometer (Bao, 2000). The amount of boron was measured using dry-ashing and an azomethine-h colorimetric method (Bao, 2000).

Treatments. On the basis of the correlation between gummosis and mineral elements that was determined from the 2018 analysis, the gummosis correlated with nitrogen, calcium, and boron, whereas the degree of gummosis negatively correlated with those of calcium and boron. In September, lesions on the grade 2 diseased trees that exhibited the strongest degree of gummosis in this area were treated. After the damaged tissue was smeared with Guoguang wound-healing agent, calcium and boron fertilizers were applied together with a base fertilizer. Three treatments were established: 1) contrast, 2) control of the application of nitrogen fertilizer (reduced up to two-thirds of the amount applied in conventional nitrogen treatments, that is, urea 0.56 kg/plant + mono-ammonium phosphate 0.86/plant), 3) 10 kg calcium superphosphate per plant, and 4) 0.05 kg borax per plant. Ten trees were established in each treatment. In the high-incidence season (September) in the second year, the statistics on number of gummosis lesions that reappeared at each treatment and the disease grade of

gummosis was documented, and the disease index and amount of relapse were calculated. Disease index = $\sum$ (each infected value * plant number at this grade) / (total plant number in this investigation * the highest infected value) * 100.

Data analysis. SPSS 18.0 (SPSS, Inc., Chicago, IL) was applied for the analysis of significant differences, binary logistic regression analysis, and ordinal logistics regression analysis. The standard for a statistical difference was $P < 0.05$. The model of binary logistic regression analysis and ordinal logistics regression analysis are as follows: grade 0 indicates trees without gummosis. Grades 1 through 4 of gummosis indicate the trees with gummosis of varying degrees. Binary logistic regression analysis was conducted. On the basis of the different degrees of gummosis, it was divided into grades 1, 2, 3, and 4. Ordinal logistics regression analysis was conducted. The level of significance of the Wald test at $P < 0.05$ presented as extremely significant and was listed as the selected factor.

Results

As shown in Table 2, there were no significant differences between the contents of iron and zinc and different grades of gummosis disease in the leaves, whereas the contents

of nitrogen, magnesium, and manganese in the plum tree leaves with gummosis were significantly higher than the contents of the trees without gummosis. Their contents in trees with a higher degree of disease were much higher. In contrast, the contents of phosphorus, potassium, calcium, and boron in the leaves of normal trees were higher than those in the trees with gummosis. Higher grades of gummosis were present in the leaves with a lower content of these minerals. As shown in Table 3, there were nonsignificant differences between the contents of phosphorus, magnesium, and iron in branches between normal trees and those with gummosis. The differences between each grade of gummosis were nonsignificant, whereas the contents of nitrogen and manganese in the branches of trees with gummosis were significantly higher than those in the normal trees. The contents of potassium, calcium, zinc, copper, and boron in the branches of normal trees were all higher than those in trees with gummosis. Higher grades of gummosis were associated with a lower content of these minerals.

As shown in Table 4, the binary logistic regression analysis on the contents of mineral elements in the leaves between the trees with and without gummosis indicated significant differences in the treatments. The factors with the significance level of $P < 0.05$ by the Wald test were listed as the selected factors. It can be seen that these factors have a significant impact on the occurrence of plum jelly disease. They were the contents of nitrogen, potassium, calcium, manganese, and boron in the leaves, as well as manganese, boron, and calcium in the branches. Therefore, the B values of nitrogen, manganese in leaves, and manganese in branches were greater than 0, and the odds ratio (OR) values were greater than 1, which indicated that the occurrence of

Table 2. Mineral element contents in plum leaves with different grades of gummosis.

Index	No gummosis	Grade 1 disease	Grade 2 disease	Grade 3 disease	Grade 4 disease
Nitrogen (g/kg)	20.13 ± 0.28 c	21.12 ± 0.21 b	21.10 ± 0.19 b	21.95 ± 0.17 a	21.25 ± 0.85 a
Phosphorus (g/kg)	2.24 ± 0.19 a	1.55 ± 0.44 b	1.59 ± 0.14 b	1.50 ± 0.03 b	1.67 ± 0.34 b
Potassium (g/kg)	25.55 ± 0.55 a	24.30 ± 0.38 b	23.27 ± 0.12 c	23.55 ± 0.03 bc	23.93 ± 0.12 c
Calcium (g/kg)	42.57 ± 1.92 a	35.40 ± 1.04 b	21.90 ± 0.29 c	23.25 ± 0.09 c	17.17 ± 0.92 d
Magnesium (g/kg)	1.92 ± 0.01 c	1.73 ± 0.18 c	2.17 ± 0.07 bc	2.86 ± 0.57 ab	3.38 ± 0.08 a
Iron (mg/kg)	66.90 ± 0.29 a	64.63 ± 4.21 a	67.05 ± 6.32 a	74.55 ± 6.55 a	75.10 ± 1.85 a
Manganese (mg/kg)	217.50 ± 34.93 d	323.00 ± 28.29 c	364.50 ± 32.02 c	463.33 ± 22.70 b	652.00 ± 23.09 a
Zinc (mg/kg)	21.60 ± 0.81 a	22.37 ± 2.86 a	25.43 ± 1.60 a	22.60 ± 1.39 a	17.35 ± 1.13 a
Copper (mg/kg)	14.65 ± 0.26 a	7.87 ± 0.55 b	8.60 ± 0.30 b	10.13 ± 0.22 b	7.20 ± 3.60 b
Boron (mg/kg)	90.93 ± 6.55 a	85.25 ± 1.07 a	53.95 ± 4.07 b	60.85 ± 2.63 b	65.55 ± 9.96 b

Different letters between treatments denote significant differences (Duncan test, $P \leq 0.05$).

Table 3. Mineral element contents in branches with different grades of plum gummosis.

Index	No gummosis	Grade 1 disease	Grade 2 disease	Grade 3 disease	Grade 4 disease
Nitrogen (g/kg)	5.56 ± 0.16 b	6.15 ± 0.06 a	6.26 ± 0.97 a	6.36 ± 0.08 a	6.46 ± 0.12 a
Phosphorus (g/kg)	1.02 ± 0.14 a	1.00 ± 0.18 a	0.98 ± 0.16 a	0.72 ± 0.02 a	0.89 ± 0.01 a
Potassium (g/kg)	5.84 ± 0.24 a	5.22 ± 0.08 b	4.83 ± 0.02 bc	4.17 ± 0.30 cd	4.35 ± 0.13 d
Calcium (g/kg)	6.74 ± 1.25 a	6.52 ± 0.18 a	5.12 ± 0.18 ab	4.14 ± 0.39 b	4.21 ± 0.54 b
Magnesium (g/kg)	0.62 ± 0.02 a	0.68 ± 0.05 a	0.65 ± 0.06 a	0.60 ± 0.10 a	0.59 ± 0.07 a
Iron (mg/kg)	29.75 ± 1.95 a	38.40 ± 4.60 a	41.35 ± 4.85 a	40.00 ± 15.60 a	43.75 ± 9.85 a
Manganese (mg/kg)	79.35 ± 14.85 c	108.67 ± 8.50 bc	145.00 ± 12.00 ab	179.00 ± 11.00 a	178.00 ± 50.69 a
Zinc (mg/kg)	20.80 ± 4.30 a	13.97 ± 3.90 b	10.05 ± 3.43b	12.94 ± 4.46 b	8.48 ± 0.82 b
Copper (mg/kg)	7.06 ± 0.52 a	6.00 ± 0.61 b	5.87 ± 0.59b	5.51 ± 0.34 bc	4.83 ± 0.32 c
Boron (mg/kg)	30.23 ± 0.70 a	26.02 ± 4.64 b	26.00 ± 0.50b	17.15 ± 0.85 c	13.38 ± 0.88 c

Different letters between treatments denote significant differences (Duncan test, $P \leq 0.05$).

P. salicina gummosis positively correlated with the contents of nitrogen and manganese in the leaves. However, the B values of the contents of potassium, calcium, and boron in the leaves and calcium and boron in branches were all smaller than 0, and the OR values were also less than 1, which indicated that the occurrence of *P. salicina* gummosis negatively correlated with them. In addition, other factors (phosphorus, iron, zinc, and copper in the leaves; nitrogen, phosphorus, potassium, iron, zinc in the branches, and copper in the branches) were not included, indicating that there were nonsignificant effects of these factors on the occurrence of gummosis in this region.

As shown in Table 5, the selected factors through binary logistic regression analysis were used to conduct ordinal logistics regression analysis. The results of ordinal logistics regression analysis between the contents of mineral element and a different degree of occurrence of gummosis were obtained. It showed that the contents of nitrogen and manganese in the leaves and manganese in the branches significantly correlated with the severity of occurrence of gummosis. The B values of the factors described earlier were larger than 0, indicating that the higher the contents of nitrogen and manganese in the leaves and manganese in the branches, the more serious the occurrence of *P. salicina* gummosis in this region. P value of regression analysis of the three factors (potassium in the

leaves, calcium in the leaves, and calcium in the branches) were greater than 0.05, indicating that the three factors are not independent risk factors for the severity of plum gum disease in this area. P values of regression analysis of boron in leaves and branches were less than 0.05, and the B value was less than 0, indicating that the content of boron in leaves and branches were significantly correlated with the severity of the disease, and the lower the content of boron in leaves and branches, the higher the severity of the disease.

As shown in Table 6, the effects of the prevention and control on *P. salicina* gummosis after fertilization treatment were apparent. Scraping the lesion and using sealing compound had some curative effects on the lesions. Therefore, after the control of nitrogen fertilizer and increase in the application of boron and calcium fertilizers, both the disease index and rate of recurrence of the lesions were lower than those of the control, indicating that after the lesions were scraped, the control of nitrogen fertilizer or increased application of boron and calcium fertilizers had some curative effects on the prevention and control of *P. salicina* gummosis in this region.

Discussion

Gummosis is a disease that severely affects *P. salicina* and other stone fruit trees. Multiple factors are involved in the progression of this disease, and it occurs in all the production

regions of peach, cherry, lemon, and citrus in all over the world (Li et al., 2016). This disease primarily damages the branches and trunks of trees, leading to gummosis in the main trunk, main branch, and other branches, resulting in large numbers of lesions in the trunk and branches that weaken the tree vigor, shorten its life, and may cause death (Li et al., 2014). Gummosis has been a large obstacle to the planting of plums. There are generally two hypotheses for the pathogenesis of gummosis in stone fruits, including infectious gummosis and physiological gummosis. The pathogenesis of physiological gummosis is generally considered to be related to the variety cultivated, tree age, physical injury, temperature and humidity, and mineral nutrition. In this study, through research on the relationship between mineral elements and the occurrence of gummosis, we identified correlations between nitrogen, calcium, and boron with the occurrence and degree of occurrence of gummosis.

Hummel et al. (2013) indicated that high nitrogen conditions were conducive to the occurrence and spread of this disease. Nam et al. (2006) and Smith (2008) hypothesized that the overuse of nitrogen fertilizer would decrease the ability of host to against this disease. In this study, we found that the content of nitrogen in the leaves significantly correlated with the occurrence of *P. salicina* gummosis and that gummosis was more prevalent when nitrogen content was higher. By controlling the application of nitrogen fertilizer, the rate of recurrence of gummosis decreased compared with the trees subjected to regular fertilization.

Calcium is involved in the generation of cell walls, modification of membrane structure, reduction in membrane permeability, delay in the time of degradation of cell wall materials, and protection of cell wall structure. Calcium treatment also suppressed the degradation of water-insoluble pectin in fruits and facilitated an increase in water-soluble pectin (Cybulska et al., 2011; Matoh and Kobayashi, 1998; Yamazaki and Hoshina, 1995). Boron was bound in the cell walls, and B-RG-II type polysaccharides were formed, which resulted in pectin nets that were crossly linked. Boron can maintain the integrity and stable cell wall structure, as well as maintain some mechanical strength and plastic stretching capacity (Dixon et al., 1973). In this study, the contents of both calcium and boron in both the leaves and branches significantly correlated with the occurrence of gummosis in the main trunk of *P. salicina*, whereas the contents of boron in leaves and branches significantly correlated with the severity of gummosis occurrence. In addition, through the application of calcium and boron fertilizers to the soil, the rate of recurrence of gummosis was lower than that under regular fertilization, indicating that the appropriate application of calcium and boron had some effects on preventing and controlling gummosis in *P. salicina*. This is consistent with the results of Mostowfizadeh-Ghahamfarsa et al. (2018), who found that the application of calcium fertilizer can reduce

Table 4. Binary logistic regression analysis results of plum gummosis and mineral element content.

Selected factors	B	SE	Wald	df	P	OR	95% CI
Nitrogen in leaves	81.85	32.65	6.29	1	0.01	3.53	0.02–0.95
Potassium in leaves	–4.71	1.96	5.79	1	0.02	0.01	0.00–0.42
Calcium in leaves	–2.37	1.01	5.53	1	0.02	0.09	0.01–0.67
Manganese in leaves	0.08	0.04	3.80	1	0.05	1.08	1.00–1.18
Boron in leaves	–0.25	0.11	5.78	1	0.02	0.78	0.63–0.95
Calcium in branches	–0.93	0.33	7.90	1	0.01	0.40	0.21–0.76
Manganese in branches	5.08	22.69	0.10	1	0.04	1.86	0.00–5.85
Boron in branches	–0.73	0.29	6.19	1	0.01	0.48	0.27–0.86

B = the regression coefficient; df = degrees of freedom; OR = odds ratio; CI = confidence interval.

Table 5. Ordered logistic regression analysis results of severity of plum gummosis and mineral element content.

Selected factors	B	SE	Wald	df	P	95% CI
Nitrogen in leaves	167.15	74.96	4.97	1	0.03	20.00 to 314.07
Potassium in leaves	–15.48	10.76	2.07	1	0.15	–36.56 to 5.61
Calcium in leaves	–2.03	8.15	0.06	1	0.80	–17.99 to 13.94
Manganese in leaves	0.07	0.02	13.67	1	0.01	0.03 to 0.10
Boron in leaves	–0.34	0.15	5.05	1	0.03	–0.63 to –0.04
Calcium in branches	–3.37	1.89	3.17	1	0.08	–7.08 to 0.34
Manganese in branches	0.07	0.02	20.84	1	0.01	0.04 to 0.10
Boron in branches	–2.25	1.09	4.25	1	0.04	–4.38 to –0.11

B = the regression coefficient; df = degrees of freedom; CI = confidence interval.

Table 6. The control effect of different treatments on plum gummosis.

Treatment	Disease index (%)	No. of spots	No. of recurrent spots	Recurrence rate (%)
Contrast	47.50	149	89	59.73
Treatment of reduced nitrogen application	32.50	139	65	45.32
Treatment with borax	30.00	138	52	37.68
Treatment with calcium fertilizer	27.50	145	41	28.28

the flow gum of pistachio. It is also consistent with the research by Nartvaranant et al. (2002) that the appropriate supplement of boron was conducive to controlling the occurrence of gummosis during the process of mango planting and management.

Manganese is involved in the process of photosynthetic oxygen evolution and the reactions of enzyme systems. It plays an important role in plant growth and development (Ferroni et al., 2004). However, in this study, the contents of manganese in leaves and branches had significant effects on the severity of occurrence of *P. salicina* gummosis with a correlation between the content of manganese and the degree of gummosis. Currently, there are some reports of research on the relationship between an excess of manganese and plant disease. Ye et al. (2002) found that the crude skin disease of apple was closely related to the excessive manganese content. Buttner and Kehr (2003) found that the extreme uptake of manganese causes the great damage to douglas fir trees. However, reports that the excessive absorption of manganese in stone fruit trees readily results in the occurrence of gummosis are rare. Questions that merit further study include whether the excessive absorption of manganese will cause gummosis in other stone fruit trees and whether the pathogenic mechanism is owing to the suppression of absorption of other elements or a direct cause of manganese toxicity. Significantly, the content of manganese in the soil of this region is not excessive, whereas the manganese content in leaves is clearly excessive. Thus, we hypothesized that it could be a result of the overuse of pesticides that contain manganese. Whether the overapplication of pesticides containing manganese in production will induce gummosis merits further study.

Conclusions

There is an important relationship between the occurrence of gummosis disease and the content of mineral elements in plants. For example, the degree of gummosis in *P. salicina* is greater when the nitrogen content in the leaves is higher. There was a significantly negative correlation between the contents of calcium and boron in the branches and leaves and the occurrence of gummosis. The grade of gummosis is higher when the contents of these elements are lower. The fertilization experiment indicated that controlling nitrogen and increasing the application of boron and calcium fertilizers have some effects on the prevention and control of gummosis in *P. salicina*. Further research on the pathogenic mechanism of the effects of excessive manganese absorption on gummosis is warranted.

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