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Control of Peronophythora fruit downy blight of lychee by neutralized phosphorous acid

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ABSTRACT

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Field studies were conducted to evaluate the effect of neutralized phosphorous acid solution (NPA) on the control of fruit downy blight of lychee (*Litchi chinensis*) caused by *Peronophythora litchii* during 1999-2003. Trees of lychee, variety 'Black Leaf', were sprayed with NPA at 1000 mg/L, 2 or 3 times at 7-day intervals, at the fruit maturing stage and then inoculated with sporangial suspension of *P. litchii* at 200-500 spores/mL. Matured fruits were harvested and percentage of downy blight fruit was recorded. Results of the 3-year field trials showed that lychee trees sprayed with NPA significantly (P<0.01) reduced the incidence of fruit downy blight (0.5-11.8%), compared to untreated controls (26.1-46.6%). The treatment of NPA also was as effective as the treatment of mancozeb 80WP (dilution 500 times), a commercial synthetic chemical registered for the control of lychee fruit downy blight in Taiwan. Meanwhile, three applications of NPA in the fields not only significantly (P<0.01) reduced the incidence of postharvest Peronophythora fruit rot of lychee (0-16%), compared to the untreated control (12.2-96.6%), but also delayed the development of postharvest fruit downy blight by 2-4 days. However, treatment of freshly harvested lychee fruits with NPA in the laboratory did not significantly (P>0.05) reduce the incidence of Peronophythora downy blight of lychee fruits during postharvest stage. These results of field and laboratory experiments indicate that NPA must be applied to lychee plants at fruit development or maturing stage in the fields to induce resistance of lychee fruits against *P. litchii* and, thereby, reduce downy blight of lychee fruits in the field and at postharvest stage.

Keywords: Lychee, *Litchi chinensis*, Lychee fruit downy blight, *Peronophythora litchii*, Disease control, Phosphorous acid, Induced resistance

INTRODUCTION

Phosphorous acid (H_3PO_3) and Phosphonate compounds have been used since 1980s for the control of diseases of field crops caused by Oomycetes [(8)]. In Taiwan, a neutralized phosphorous acid solution (NPA) at pH 6.2-6.7 was developed and used for the control of Phytophthora diseases in the fields [(2,3,4)]. The NPA solution was prepared by dissolving phosphorous acid in water and then adding an equal weight of potassium hydroxide to the solution. Previous studies showed that the NPA solution was easy to prepare and was effective in the control of late blight of potato and tomato caused by *Phytophthora infestans*

(Montagne) de Bary [14], leaf and blossom blight of lily caused by *P. nicotianae* Breda de Haan (= *P. parasitica* Dastur) [3], basal stem rot of pepper caused by *P. capsici* Leonian [3] and seedling root rot of avocado caused by *P. cinnamomi* Rands, fruit rot of and leaf blight of kumquat caused by *P. citrophthora* Leonien and basal stem rot and root rot of loquat caused by *P. nicotianae* [4].

In Taiwan, lychee (*Litchi chinensis*) (also called litchi) is an economically important fruit-tree crop. One of the major factors limiting production of lychee fruits is the disease of fruit downy blight (also called Peronophythora fruit rot or Peronophythora downy blight) caused by *Peronophythora litchii* Chen ex Ko *et al.* [7,12]. The pathogen could ruin almost all of ripening fruits in lychee orchards, especially under high humidity caused by continuing rainfalls. Since *P. litchii* is a member of Oomycetes and there were no previous reports on the control of fruit downy blight of lychee by phosphorous acid, the objective of this study was to determine effectiveness and practicality of using NPA for the control of fruit downy blight in lychee orchards.

MATERIALS AND METHODS

Preparation of phosphorous solution

Neutralized phosphorous acid (NPA) solutions were prepared by the method of Ann *et al.* [3], using high purity phosphorous acid (H_3PO_3, 99% white crystal, Aldrich Co., USA) and experimental grade potassium hydroxide (≥85% KOH pellet GR, Merck Co., USA). The procedure was dissolving phosphorous acid into distilled water first and then adding an equal weight (1:1 ratio, w/w) of potassium hydroxide to the phosphorous acid solution. Ann *et al.* [3] reported that pH values of NPA solutions at 500-5000 mg/L were 6.26-6.45. The prepared NPA solution was applied to test plants (lychee trees and fruits) immediately. In the preliminary tests, the NPA exceeding 1000 mg/L was phytotoxic, causing skin browning of lychee fruits. Therefore, the concentration of NPA at 1000 mg/L was used for spraying on lychee trees at fruit maturing stage in this study.

Pathogen and of inoculum preparation

Peronophythora litchii used in this study was a single-zoospore, homothallic strain PL5-1 (TARI98125) isolated by the first author from a diseased lychee fruit collected at Caotun, Nantow county in 1998. Stock cultures were prepared using the modified method of Boesewinkel [5] by adding 5% V-8 juice agar blocks containing mycelial mats into sterile distilled water in test tubes and storing the cultures at 20-24°C. The 5% vegetable juice agar (VA) contained 5% V-8 juice (Campbell Co. USA), 0.02% $CaCO_3$ and 1.5% agar (Hwei Shen Co., Taiwan)

To prepare working cultures, *P. litchii* in stock cultures was transferred to fresh 5% VA and incubated at 24°C for 3-5 days. An agar disc (av. 0.5 cm × 0.5 cm × 0.3 cm) containing mycelial mats was removed from the periphery of 3-5-day-old colony and placed at the center of a Petri dish (9 cm diam.) containing fresh 10% VA. Numerous sporangia were formed after the cultures were incubated at 24°C under continuous fluorescent light at 3000 Lux for 5 to 7 days. The culture in each dish was sprayed with 20 mL sterile distilled water to wash off sporangia and the solution was filtered through double-layer sterile cheesecloth with the aid of an automizer to collect sporangia suspension. The sporangia suspension was adjusted to about 200-500 spores/mL and used for inoculation of lychee fruits.

Control of Peronophythora fruit rot of lychee by NPA and synthetic fungicides in the fields

Field experiments were conducted in two lychee orchards, one located at Fenyuan, Changhwa in 2000 and another located at Caotun, Nantow in 2001 and 2002. Ten to fifteen-year-old lychee trees, var. ‘Black-leaf‘, were used for the experiments. Treatments were NPA applications (2 and/or 3 times) and untreated control, one or two plants per treatment; and there were 3 replicates per treatment arranged by randomized complete block design (RCBD). During fruit maturing stage, each lychee tree was sprayed with 20 L of NPA at 1000 mg/L, 2-3 times at 7-day intervals. Seven days after last chemical spray, lychee fruits turning green-red color were selected for inoculation with *P. litchii* by spraying 10 mL sporangial suspension on each fruit bunch, 20

bunches per treatment. Each fruit bunch was covered with a plastic bag (60 cm × 60 cm, L x W) and tied with a string. Each fruit bunch of lychee in the plastic bag was harvested 7 days after inoculation and the number of fruits infected by *P. litchi* were recorded for calculation of incidence (%) of downy fruit rot. Each treatment consisted of 20 bunches of lychee fruits from one or two trees and had 3 replicates. The experiment was repeated for 3 years. Fruits on lychee trees without chemical application but inoculated with *P. litchii* were covered with plastic bags and used as controls.

To compare the effects of NPA with other fungicides in the control of lychee fruit downy blight, lychee plants sprayed with 80% Mancozeb WP (Rohm and Haas Co., Ltd. in Taiwan) diluted 500 times (conc. ai 1600 mg/L) or 50% Prochlorate manganese WP (Aige Fu Co., Ltd. in Taiwan) diluted 4000 times (a.i. 500 mg/L) in 2001 were inoculated with sporangial suspension of *P. litchii* and fruits were harvested and examined for the incidence of fruit rot by the same method described in the NPA treatment.

Control of postharvest fruit rot of lychee by NPA in the fields

To understand the effect of field application of NPA on the control of lychee fruit downy blight at postharvest stage, NPA was sprayed on lychee trees at fruit maturing stage in the fields in 1999 at Fenyuan, Changhwa, and in 2001, 2002 and 2003 at Caotun, Nantow. The lychee variety and NPA application methods and schedules were the same as those described previously. When lychee fruits turned red and matured, they were harvested from the trees in the fields, washed with tap water, air-dried for 30 min, dipped in the sporangial suspension of *P. litchii* at concentration of 200-500 sporangia/mL for 5 min, placed on moist paper in plastic containers (35 cm × 25 cm × 10 cm), and incubated at 24℃. Disease incidence was recorded by daily examination of downy fruits rot for 7 days, based on the tests of 50-100 fruits. For the control treatment, lychee fruits from trees without treatment of NPA in the field were harvested, inoculated with *P. litchi* in the laboratory and examined for the incidence of fruit rot. Each treatment consisted of 50 to 100 fruits with 3 replicates. The study was repeated 7 times.

Control of Peronophythora fruit rot of lychee by postharvest treatment with NPA

The effect of postharvest treatment of lychee fruits with NPA on the control of fruit downy blight was also evaluated. Fruits without pre-treatment with NPA in the fields were harvested from an orchard at Caotun, Nantow in 2002. They were washed, air-dried, dipped in NPA solution at 1000 mg/L for 5 min, air-dried for 30 min, inoculated with sporangial suspension of *P. litchii* (200-500 spores/mL) for 5 min and then incubated in moist containers at 24°C for 7 days. Fruits without treatment of NPA but inoculated with *P. litchii* were used as controls. Disease incidence was recorded daily for 7 days. Each treatment consisted of 50-100 fruits with 3 replicates. The study was repeated once.

Statistical analysis

Data collected from each experiment were analyzed by analysis of variance (ANOVA), and means were compared by least significant difference (LSD) test at P=0.05 or P=0.01.

RESULTS

Control of Peronophythora downy blight of lychee fruits with NPA in the fields

Results of the three-year field experiment in lychee orchards in 2000-2002 showed that application of NPA solution (1000 mg/L) on lychee trees, two to three times at 7-day intervals, significantly (P<0.01) reduced the incidence of Peronophythora fruit downy blight of lychee (Table 1). For example, the incidence of fruit downy blight of lychee in 2000, 2001 and 2002, in the treatment of three applications of NPA was 7.3, 6.7 and 0.5%, respectively, compared to 26.1, 36.4 and 46.6% in the untreated controls. Application of NPA two times in the field was also effective and the incidence of fruit rot in 2000 was 11.8% in the treatment of NPA, compared to 26.1% in the untreated control. Although 3 applications of NPA was more effective than 2 applications, the difference in fruit rot incidence was insignificant (P>0.05) between two and three applications.

Results of the field experiments in 2001 and 2002 also showed that all the chemicals, NPA (1000 mg/L), 80% mancozeb WP (1600 mg/L) and 50% Prochlorate manganes WP (500 mg/L), applied 3 times on lychee trees in the orchards at 7-day intervals were effective in the control of fruit downy blight of lychee (Table 2). The efficiency in reducing fruit downy blight by NPA (1000 mg/L) was the same as the synthetic chemical 80% mancozeb WP (1600 mg/L). In 2001, the incidences of fruit downy blight of lychee in the treatments of NPA, mancozeb and Prochlorate manganes was 6.7, 10.6 and 19.8%, respectively, compared to 36.4% in the untreated control. Results of the field experiment in 2002 were similar to those in 2001 (Table 2).

Effect of treatment of lychee plants with NPA in the fields on control of Peronophythora fruit rot of lychee fruits at postharvest stage

Lychee fruits were harvested from trees sprayed with NPA at 1000 mg/L, 2-3 times at 7-day intervals in the orchards and tested for control of Peronophythora fruit rot at postharvest stage, 7 times during the 4-year period in 1999, 2001, 2002 and 2003. Results showed that compared to the untreated controls, lychee plants treated with NPA at fruit maturing stage in the fields significantly (P<0.01) reduced the incidence of downy fruit rot of lychee after harvest (Table 3). Meanwhile, NPA applied to lychee fruits in the fields also delayed the development of fruit downy blight by 2-4 days, compared to the untreated controls (data not shown).

Control of Peronophythora fruit rot of lychee by postharvest treatment with NPA

Results of the laboratory experiments in 2002 showed that postharvest treatment of lychee fruits with NPA was ineffective in the control of Peronophythora fruit downy blight (Table 4). All harvested lychee fruits treated with NPA and inoculated with *P. litchii* or the control (inoculated with the pathogen without pre-treatment of NPA) developed symptoms of downy fruit rot in 2-3 days after inoculation of the pathogen.

DISCUSSION

Fruit downy blight caused by *P. litchii* is an important disease of lychee in Taiwan [7,12]. This disease has existed in Taiwan for more than 70 years and it is particularly severe in rainy season because humid climates are favorable for propagation, dissemination and infection of the pathogen (P. J. Ann, unpublished data). Under continuing rainfalls which occurred frequently in May-July, *P. litchii* could ruin almost all of the maturing and matured fruits in the lychee orchards with heavy infestation of the pathogen.

Several synthetic fungicides have been registered for commercial use in the control of fruit downy blight of lychee in the fields in Taiwan [9], including mancozeb, oxine-copper,

Table 1. Control of Peronophythora fruit downy blight of lychee by neutralized phosphorous acid solution (NPA)[a] in the fields

	Incidence of infected fruits[c] (%)		
Treatment[b]	2000	2001	2002
NPA , 2 sprays	11.8 a[d] (54.8)[e]	NT[f]	NT
NPA , 3 sprays	7.3 a (72.0)	6.7 a (81.6)	0.5 a (99.1)
Control	26.1 b	36.4 b	46.6 b

[a] 1000 mg/L of H_3PO_3 solution was neutralized with equal weights of KOH. Both chemicals are experimental grade.

[b] Trees of lychee var. 'black leaf' were sprayed with NPA at 7-day intervals during fruit maturing stage.

[c] Seven days after the last H_3PO_3 application, fruits covered with plastic bags, were inoculated with sporangial suspension (200-500 spores/mL) of *Peronophythora litchii* in the fields, and disease was assessed 7 days after inoculation.

[d] Means followed by the same letters in the same year were not significantly different at 1% level according to LST test.

[e] Data in parenthesis refer to disease reducing rates (%).

[f] NT=not tested.

Table 2. Control of Peronophythora fruit downy blight of lychee in the fields by neutralized phosphorous acid solution (NPA)[a] and other synthetic chemicals

Chemical [b]	Concentration (mg/L)	Incidence of infected fruits (%)[c]	
		2001	2002
NPA	1000	6.7 a[d] (81.6)[e]	33.0 a (59.1)
80% Mancozeb WP	2000	10.6 a (70.8)	30.0 a (62.8)
50% Prochlorate manganese WP	500	19.8 b (45.6)	62.0 b (23.1)
Control (water)		36.4 c	80.7 c

[a] 1000 mg/L of H_3PO_3 solution was neutralized with equal weights of KOH. Both chemicals are experimental grade.

[b] For each chemical treatment, trees of lychee var. 'black leaf' were sprayed with chemical, 3 times at 7-day intervals, during fruit maturing stage.

[c] Seven days after the last chemical application, fruits in the fields were inoculated with sporangial suspension (200-500 spores/mL) of *Peronophythora litchii*, covered with plastic bags for 7 days, and examined for disease incidence.

[d] Means followed by the same letters in the same year were not significantly different at 1% level according to LST test.

[e] Data in parenthesis refer to disease reduction rates (%)

Table 3. Effect of application of neutralized phosphorous acid solution (NPA)[a] in the field on control of Peronophythora downy blight of harvested lychee fruits.

Treatment[b]	Incidence of infected fruits[c] (%)						
	1999-1	1999-2	2001	2002-1	2002-2	2003-1	2003-2
NPA, 2 sprays	8.1 a[d]	16 a	NT[d]	NT	NT	NT	NT
NPA, 3 sprays	NT	NT	0 a	7.1 a	0 a	0 a	0 a
Control	12.2 b	55 b	25 b	96.6 b	42 b	12.2 b	59.0 b

[a] 1000 mg/L of H_3PO_3 solution was neutralized with equal weights of KOH. Both chemicals are experimental grade.

[b] Trees of lychee var. 'Black-leaf' were sprayed with NPA at 7-day intervals during fruit maturing stage.

[c] The harvest lychee fruits were inoculated with *Peronophythora litchii* by dipping fruits in a suspension of sporangial (200-500 spores/mL) for 5 min, placed on moist paper in plastic containers, incubated at 24°C for 3 days and examined for disease incidence.

[d] Means followed by the same letters in the same year were not significantly different at 1% level according to LST test.

[e] NT=not tested.

Table 4. Effect of postharvest treatment with NPA[a] on control of Peronophythora fruit downy blight of lychee

Treatment	Incidence of infected fruits (%)[2] at the day after inoculation					
	1st	2nd	3rd	4th	5th	6th
NPA	0	7.5 a[c]	73.0 a	100	100	100
Control	0	10.5 a	69.3 a	100	100	100

[a] 1000 mg/L of H_3PO_3 solution was neutralized with equal weights of KOH. Both chemicals are experimental grade.

[b] Mature fruits of lychee var. 'Black-leaf' were harvested, dipped in NPA solution for 5 min, air-dried for 30 min, and then dipped in a suspension of sporangia (200-500 spores/mL) of *Peronophythora litchii* for 5 min. The inoculated fruits were placed on moist paper in plastic containers, incubated at 24°C for 7 days and examined for disease incidence.

[c] Means followed by the same letters on the same tests were not significantly different at 5% level according to LST test.

mancozeb + cymoxanil, dithanon + copper hydroxide, kasugamycin + carbendazin and fosetyl-aluminium + oxine-copper**.** However, control of fruit diseases with synthetic fungicides at fruit maturing stage prior to harvest is prohibited for safety reason. Therefore, searching alternative materials that are effective, safe, cheap and easy to use for the control of Peronophythora downy blight of lychee fruits have become a major task for researchers in Taiwan. Results of this study reveal that NPA sprayed on lychee trees at fruit maturing stage is effective in the control of Peronophythora downy blight of lychee fruits in the fields and at postharvest stage. Since the use of mancozeb 80WP (dilution 500 times) for control of downy blight of lychee fruits is prohibited at fruit harvest stage due to potential risks of chemical residues on fruits, this study reveals that NPA is an ideal alternative to mancozeb 80WP for practical control of *P. litchii* because it is effective, safe, cheap and easy to use.

Phosphorous acid and its salt-base compounds have been reported as effective chemicals for control of crop diseases caused by *Phytophthora* species and other Oomycetes in many countries [8,11]. Saindrenan and Guest [13] indicated that phosphorous acid ion could induce host resistance against pathogen infection as the major mechanism for control of diseases by these chemicals. In this study, NPA is effective in the control of downy blight of lychee fruits in the fields and in storage only when the chemical is applied in the fields, but is ineffective by the treatment of lychee fruits with NPA after harvest. These results indicate that induced resistance to *P. litchii* is also the mechanism for effective control of downy blight of lychee by NPA.

Many reports indicated that Phosphorous acid and its salt-base compounds could reduce severity and incidence of some fungal diseases such as melanose of citrus and bacterial diseases such as brown spot of citrus [1]. Currently, several products of phosphorous compounds have been developed and used commercially for control of Oomycete diseases [6,10,15]. However, none of these products are commonly used in Taiwan due to high price and easy oxidation in air. An alternative method for the direct use of phosphorous acid was developed in TARI by neutralizing phosphorous acid with an equal weight of potassium hydroxide in water (NPA) [2]. The NPA solution is cheap and easy to prepare. The NPA in this study may be a viable alternative for practical control of fruit downy blight of lychee as it is effective, easy to use and affordable.

In our previous reports, NPA was effective in the control of Phytophthora diseases of vegetable, flower and fruit seedling crops, including late blight of potato and tomato caused by *P. infestans* [14], basal stem rot of pepper caused by *P. capsici* [3], foliar and blossom blight of lily caused by *P. nicotianae* [3], and seedling foot and root rot of avocado, kumquat and loquat caused by *P. cinnamomi, P. citrophthora,* and *P. nicotiana*e [4] . This study shows the effectiveness and usefulness of NPA for the control of Peronophythora downy blight of lychee fruits caused by *P. litchii* in the fields and at postharvest. This study also indicates that NPA must be applied on lychee trees in the fields at fruit development stage for effective prevention of downy blight of lychee fruits. Besides lychee, this study can be a model of using NPA for control of fruit rot of other crops caused by *Phytophthora* species and other Peronospoaceae, in both conventional and organic fruit production because of no residue problems on harvested fruits.

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摘 要

安寶貞[1,3]、蔡志濃[1]、楊宏仁[2]. 2011. 利用中和後之亞磷酸防治荔枝果實露疫病. 植病會刊 20: 90-97. ([1]台中市霧峰區 農業試驗所植物病理組；[2]嘉義市 農業試驗所嘉義分所；[3]聯絡作者，電子郵件：pjann@tari.gov.tw；傳真：+886-4-2330-2803)

近年來，農試所研發一種簡單的方法配製亞磷酸中和液 (NPA)，將等重量之氫氧化鉀加入亞磷酸水溶液中和後使用。於 1999-2003 在田間施用 NPA，以評估其對荔枝果實露疫病的防治效果。在荔枝黑葉果實近成熟期，每隔 7 天噴布濃度 1000 mg/L 之 NPA 一次，共 2-3 次，相隔 7 天之後，再接種露疫病菌孢囊懸浮液（濃度為 200-500 spores/mL）。結果顯示，NPA 在田間防治荔枝果實露疫病之效果非常好，果實發病率可從 26.1-46.6%下降為 0.5-11.8%。同時，NPA 防治果實露疫病的效果與噴施三次 80% 鋅錳乃浦可濕性粉劑（稀釋 500 倍）的效果一樣好，無顯著性差異。此外，人工接種之結果顯示，在田間施用相同濃度的 NPA 2-3 次，同樣可以顯著降低採收後果實露疫病之發病率，從 12.2-96.6% 下降至 0-16%；還可以延緩果實露疫病病徵之出現達 2-4 日。然而，以相同濃度之 NPA 處理採收後的果實再接種露疫菌，則無法顯著降低果實之發病率，此項結果顯示，NPA 必須施用於田間才有防治荔枝果實露疫病之功效。

關鍵詞：荔枝果實露疫病、*Peronophythora litchii*、病害防治、亞磷酸、誘導抗病