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Control of Chinese Leek Rust with a Plant Nutrient Formulation

Jenn-Wen Huang

Department of Plant Pathology, National Chung Hsing University, Taichung 40227, Taiwan, Republic of China
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ABSTRACT

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Application of CH100, a formulation of plant nutrients, was effective in reducing the severity of rust (*Puccinia allii*) in Chinese leek (*Allium odorum*). Germination of urediniospores of the pathogen was inhibited by CH100, especially at high pH. Among the eight major ingredients of CH100 tested, calcium chloride, calcium nitrate, calcium oxide and beef extract inhibited urediniospore germination of *P. allii*, and calcium chloride and calcium nitrate reduced disease severity. CH100 stimulated proliferation of yeast population, especially *Rhodotorula* spp. and *Cryptococcus* spp. on leaf surfaces of Chinese leek. Isolates of *Rhodotorula* spp. such as YCR-089, YCR-093, and YCR-099 and *Cryptococcus* spp. such as YCP-022, YCP-046, YCP-058, YCP-211, and YCP-215 at 10^2 to 10^6 cfu/ml significantly inhibited urediniospore germination of the pathogen. Furthermore, *R. glutinis* (isolate YCR-099) was able to increase slightly inhibition of urediniospore germination by a 300-fold dilution of CH100. Chinese leek sprayed three times with CH100 at a concentration of 10 ml/L before inoculation with urediniospores of the pathogen had significantly fewer germinated urediniospores and appressoria on stomata. Chinese leek sprayed three times with CH100 had higher leaf vein ridges and narrower inter-ridge furrows than nontreated plants. The study suggests control of *P. allii* by CH100 is due to several factors including suppressive effects to the pathogen by the chemicals in CH100, increased antagonistic microbial populations on leaf tissues such as *Rhodotorula* spp. and *Cryptococcus* spp., changed leaf surface structure to reduce infection by the pathogen.

Key words: Chinese leek rust, yeasts, control.

INTRODUCTION

Rust caused by *Puccinia allii* (DC.) Rud. is a serious disease of Chinese leek (*Allium odorum* L.) in central Taiwan (13,19). Four fungicides are registered for the control of the disease, however, there is concern about the potential harmful effects on human health. As an alternative to fungicides, manipulation of crop nutrition has shown promise for disease management (11,18). Disease severity can be affected by nutrient elements which change population dynamics of microorganisms including plant pathogens and/or alter host resistance to plant pathogens (3). Blakeman and Brodie (5) reported that higher levels of major nutrients enhanced activity of epiphytic microorganisms and prolonged their survival on leaves. Also, stimulation of activity of yeast populations on phyllosphere by nutrient treatment can provide biocontrol of some plant diseases (4,7,17). McLaughin *et al* (16) reported that calcium salts dramatically improved the efficacy of antagonistic yeast strains used for control of Botrytis rot of apple.

A plant nutrient formulation, CH100, obtained from a mixture of organic and inorganic materials fermented in Hoagland's solution has effectively controlled *P. allii* in Chinese leek in both greenhouse and field tests (9). Spraying Chinese leek with CH100 increased leaf surface yeast flora, especially *Rhodotorula* spp. (10). Furthermore, spraying with CH100 increased the potassium and calcium contents of leaves of Chinese leek (10). However, the exact mechanisms of suppression of the pathogen were not identified. The purpose of the experiments reported here was to investigate the characteristics and the mechanisms of suppression of *P. allii* in Chinese leek treated with CH100.

MATERIALS AND METHODS

CH100 formulation

CH100 was prepared as described by Huang (9). A mixture containing 44 kg chopped fresh but senescent cabbage leaves, 10 kg dry tobacco debris, 5 kg $CaCl_2$, 1 kg beef extract, 30 kg S-H mixture and 200 L Hoagland's solution was mixed in a plastic tank and fermented for 45 days at 25–30 C. The fermented mixture was filtered through two 5-cm layers of sterile sponge and the filtrates were mixed with 0.5% (v/v) ethanol (95%). This stock solution of CH100 was diluted and pH adjusted for use as a foliar spray.

Host plant

Seeds of Chinese leek (*cv.* Ta-Yen) collected from Chihu, Changhua County, were sown in a 4:1 (v/v) peat:sand mixture. After 8 weeks, 25 seedlings were transplanted into plastic flats (60 × 20 × 20 cm, L × W × H) containing a similar peat:sand mixture and maintained in the greenhouse.

Inoculum of *Puccinia allii*

Inoculum of *P. allii* was prepared as described by Jennings *et al* (12). Chinese leek leaves naturally infected by *P. allii* from Chihu, Changhua County and Chingshui, Taichung County, respectively, were cut into 8-cm pieces and placed in 9-cm Petri dishes containing two moist filter papers to maintain high humidity. After incubation at 16 C in darkness for 24 hr, urediniospores were brushed from uredosori on the diseased leaves and stored at 5 C until used as inoculum.

Spore germination bioassay

Germination rate of *P. allii* urediniospores was estimated on detached leaves of Chinese leek. The fourth and fifth leaves of healthy Chinese leek were collected from the greenhouse and cut into 8-cm pieces. Two leaf pieces were mounted on a glass slide and placed in a Petri dish containing two moist filter papers. Urediniospores were suspended in distilled water and the density adjusted to 10^6–10^7 spores per ml with a hemacytometer. The detached leaf pieces were sprayed with the urediniospore suspension and incubated for 24 hr at 16 C. Leaf pieces were stained with cotton blue in lactophenol and examined to assess germination of urediniospores under a light microscope. Germination was considered to have occurred when germ tubes were at least as long as the diameter of the urediniospore. For each replicate, 100 urediniospores were examined. Four replicates were conducted per experiment.

Effect of salt and CH100 solutions on severity of rust

Inorganic salt solutions of calcium chloride ($CaCl_2$), calcium oxide (CaO), calcium nitrate [$Ca(NO_3)_2$] and potassium nitrate (KNO_3) (products of Osaka Hayashi Pure Chemical Industries Ltd., Japan), were prepared by dissolving 250 mg of each chemical in 1 L distilled water. Solutions of CH100 were prepared by mixing 10 ml of CH100 stock solution with 990 ml of distilled water and adjusting pH to 4.5, 7.7 or 8.5 by 1 N HCl or 1 N NaOH. Test solutions were sprayed on 10-week-old plants of Chinese leek once per week and for three weeks. Distilled water (pH 6.0) alone was used as the control. Four replicate flats of Chinese leek arranged in a randomized complete block design were treated with each solution in the greenhouse. One week after third spray treatment, plants were inoculated by spraying to runoff with a suspension of *P. allii* urediniospores (5.8×10^5 spores/ml) with 0.01% (v/v) Tween-20 (Osaka Hayashi Pure Chemical Industries Ltd.). Inoculated plants were incubated at 16–22 C for four weeks (with polyethylene cover during the first 48 hr) and rated for disease severity on the fourth and fifth leaves using a scale of 1 to 5, with 1 representing 1–5% diseased area and 2, 3, 4, and 5 denoting 6–15%, 16–25%, 26–50% and ≧ 51% diseased areas, respectively (10). The disease severity percentage (DSP) of inoculated plants was calculated by the formula: $DSP = [\Sigma (nR)/5 \times T] \times 100\%$, where n = number of leaves in each rust rating, R = rust rating, and T = total number of leaves. Above-ground tissues of the plants were harvested at 20 weeks old and each replicate was weighed.

Effect of CH100 with different pH values on urediniospore germination

The pH values of 100- and 300-fold dilutions of CH100 were 7.7 and 6.5, respectively. To determine whether changes in pH values of dilute solution caused CH100 to be suppressive or conducive to the pathogen, 3 ml and 10 ml of CH100 were respectively mixed with 897 ml and 990 ml of distilled water of which pH values were adjusted with 1 N HCl or 1 N NaOH to obtain CH100-diluted solutions with pH 4.5, 6.5, 7.7 or 8.5. Distilled water with the same pH values was used as the control. The effect of CH100-diluted solutions and distilled water with different pH values on urediniospore germination of the pathogen was determined by the spore germination bioassay method as described above.

Effect of major ingredients in CH100 on urediniospore germination

Urediniospores of *P. allii* from Chihu and Chingshui were mixed separately with 250 mg/L calcium chloride ($CaCl_2$), 250 mg/L calcium oxide (CaO), 250 mg/L calcium nitrate [$Ca(NO_3)_2$], 250 mg/L potassium nitrate (KNO_3), 50 mg/L beef extract (Sigma Chemical Co. St. Louis, MO), 1.5 ml/L S-H extract, 1.0 ml/L tobacco extract, 2.0 ml/L cabbage extract or 10 ml/L CH100. Detached leaf pieces of

Chinese leek were sprayed with above suspension and incubated for 24 hr at 16 C. Spore germination was assessed by light microscope after staining with cotton blue in lactophenol. Leaf pieces sprayed with urediniospores in distilled water were used as the control. Extracts of S-H or tobacco were prepared by soaking 1000 cm^3 of S-H mixture (20) or dry tobacco debris with 1 L of distilled water for 6 hr at 25 C and filtering through two layers of sterile cheesecloth. Cabbage extracts were prepared by crushing 1 kg of fresh leaves with 1 L of distilled water and filtering through two layers of sterile cheesecloth, followed by centrifugation at 4,000 *g* for 5 min and filtration through sterile Whatman No. 1 paper.

Effect of yeasts on urediniospore germination

Sixteen yeast isolates, YCP-022, YCP-035, YCP-046, YCP-058, YCP-211, YCP-215, YCP-234, YCR-051, YCR-064, YCR-077, YCR-089, YCR-090, YCR-093, YCR-099, YCW-013 and YCW-022, were obtained from leaves of Chinese leek sprayed with 300-fold dilution of CH100 for 2 days in the field (10). The isolates were cultured on glucose-yeast extract-peptone agar (GYPA)(7,10) and identified according to API 20 Aux Kit (API Co., Marcy-l'Etoile, France) and Lodder's system (15). Each yeast isolates was streaked on GYPA slants and incubated for 48 hr at 24 C. Yeast cells of each isolate were suspended in sterile distilled water. Approximately 1.0 mg of urediniospores from fields in Chingshui were mixed with 5.0 ml of each yeast suspension at 0, 10^2, 10^4 and 10^6 cfu/ml and sprayed on Chinese leek leaf pieces to assess spore germination.

Effect of major ingredients in CH100 on the growth of yeasts

The fourth and fifth leaves of healthy Chinese leek collected from the greenhouse were cut into 10-cm pieces and placed in 15-cm Petri dishes (10 g/dish) with two moist filter papers. Cells from 48-hr-old cultures of yeast isolates, YCR-099 and YCP-215, were suspended at 10^3 cfu/ml in 250 mg/L calcium chloride, 250 mg/L calcium oxide, 250 mg/L calcium nitrate, 250 mg/L potassium nitrate, 50 mg/L beef extract, 1.5 ml/L S-H extract, 1.0 ml/L tobacco extract, 2.0 ml/L cabbage extract or 10 ml/L CH100 solutions. Yeast suspensions (0.5 ml each) were sprayed on Chinese leek leaf pieces in the Petri dishes. After 48 hr incubation at 16 C, yeast populations in various treatments were assessed by putting 10 g of leaf pieces in 90 ml of distilled water in a 250-ml flask, shaking at 150 rpm for 10 min, and spreading 0.1 ml over each GYPA plate with 33 mg/L rose Bengal and 30 mg/L streptomycin sulfate (10). Yeast suspensions in distilled water were used as the control. Average number of yeast colonies was obtained from four plates of each replicate. Four replications of each treatment were conducted.

Effect of CH100 and a pink yeast on urediniospore germination

A pink yeast, *Rhodotorula glutinis* (Fres.) Harrison (isolate YCR-099), was streaked on GYPA slants and incubated for 48 hr at 24 C. Yeast cells of *R. glutinis* was suspended at 0, 10^3, 10^5, and 10^7 cfu/ml in sterile distilled water, 100-, and 300-fold dilutions of CH100. Approximately 1.0 mg of urediniospores from Chingshui area were mixed with 5.0 ml of each yeast suspension and sprayed on detached leaf pieces of Chinese leek to assess spore germination.

Effect of spraying frequency of CH100 on urediniospore germination on Chinese leek

Ten week-old plants of Chinese leek were sprayed 0-, 1-, 2-, and 3- times with 10 ml/L CH100 (pH 7.7) once per week in the greenhouse. One week after the third treatment, plants were inoculated with urediniospores (10^6–10^7 spores/ml) of *P. allii* from Chingshui area and covered with polyethylene bags for 24 hr at 16 C. Two 8 cm segments from the midsection of the fifth leaves of treated plants were mounted on a glass slide and stained with cotton blue in lactophenol. The number of germinated spores, appressoria, and appressoria on stomata were counted under a light microscope. For each replicate, 100 urediniospores were examined. Four replicates were conducted per treatment.

Effect of CH100 on leaf morphology of Chinese leek

CH100 solution was sprayed at 10 ml/L to 10 week-old plants of Chinese leek once per week in the greenhouse. Ten pieces of fifth leaves of plants sprayed 0-, 1-, 2- and 3-times were cut into 5 × 7 mm rectangles and fixed in 2% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.0) overnight at 4 C. Samples were dehydrated in graded ethyl alcohol series, critical-point dried with carbon dioxide and coated with gold in a JBS E5150 sputter coater, and examined with a Bausch & Lomb Nanolab 2100 scanning electron microscope (SEM) operated at 15 kV. Heights of 40 leaf veins, widths of 40 furrows between two leaf vein ridges, lengths and widths of 30 stomata were measured each replicate sample. Four replications were conducted per treatment.

Statistical Analyses

Data were evaluated by analysis of variance and general linear model statistical procedures with the SAS/STAT system for personal computers (SAS Institute, Inc., Cary, N. C.). Statistically significant differences among means were determined by Duncan's multiple range test.

RESULTS

Effect of salt and CH100 solutions on severity of rust

Three applications of $CaCl_2$, $Ca(NO_3)_2$, CH100 (pH 8.5) or CH100 (pH 7.7) before inoculation of *P. allii* reduced severity of Chinese leek rust by 30% compared to the control (Fig. 1). In addition, disease severity was reduced by 8 to 15% in plants treated with CH100 (pH 4.5), KNO_3, and CaO. At pH 7.7 or 8.5, CH100 was more effective in reducing disease severity than at pH 4.5. Yields of Chinese leek were significantly (P=0.05) higher after treatment with CH100 (pH 8.5), KNO_3, CH100 (pH 7.7), CaO, $Ca(NO_3)_2$, and CH100 (pH 4.5) compared to the treatments of $CaCl_2$ and control (Fig. 1).

TABLE 1. Effect of CH100 with different pH values on urediniospore germination of *Puccinia allii* on detached leaves of Chinese leek after 24 hr at 16 C

Dilution of CH100	pH values[2]	Germination (%) Exp. I	Exp. II
100	8.5	21 de[3]	22 c
100	7.7	23 d	24 c
100	4.5	60 a	53 a
300	8.5	15 de	14 d
300	6.5	14 e	16 cd
300	4.5	50 bc	52 a
D.W.[1]	8.5	47 c	44 b
D.W.	7.7	56 ab	57 a
D.W.	6.5	56 ab	54 a
D.W.	4.5	59 ab	57 a

[1] D. W.: Distilled water was used as control.
[2] pH values of CH100 and distilled water were adjusted by 1N HCl or 1N NaOH.
[3] Means (n=4) followed by the same letter are not different significantly (P=0.05, Duncan's multiple range test).

TABLE 2. Effect of CH100 and its major ingredients on urediniospore germination of *Puccinia allii* from Chihu and Chingshui areas on detached leaves of Chinese leek after 24 hr at 16 C

Treatment	Conc. (ppm)	Germination (%) Spores from Chihu	Spores from Chingshui
$CaCl_2$	250	31 de[1]	36 c
CaO	250	43 bc	42 bc
$Ca(NO_3)_2$	250	41 c	37 c
KNO_3	250	44 abc	49 ab
S-H extract	1500	52 a	51 ab
Beef extract	50	39 cd	45 bc
Cabbage extract	2000	51 ab	58 a
Tobacco extract	1000	52 a	50 ab
CH100	10000	23 e	24 d
Distilled water (CK)	—	50 ab	55 a

[1] Means (n=4) within the column followed by the same letter are not significantly different (P=0.05, Duncan's multiple range test).

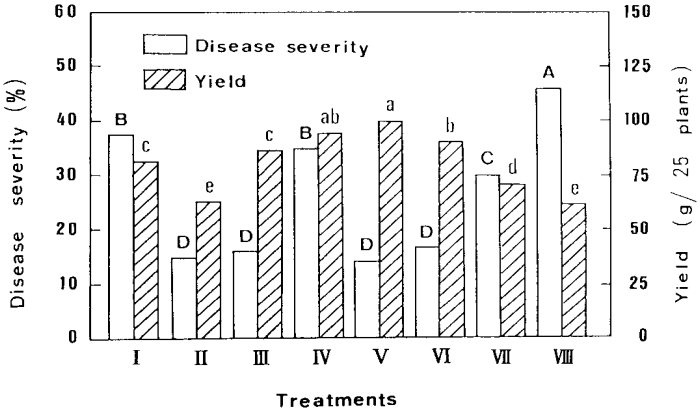


Fig. 1. Effect of salt and CH100 solutions on severity of Chinese leek rust caused by *Puccinia allii* in the greenhouse at 16–22 C. (I: CaO; II: $CaCl_2$; III: $Ca(NO_3)_2$; IV: KNO_3; V: CH100 (pH 8.5); VI: CH100 (pH 7.7); VII: CH100 (pH 4.5); and VIII: Distilled water (Control)). Means (n=4) (Columns) for disease severity or fresh yield with the same letter do not differ significantly (P=0.05, Duncan's multiple range test).

Effect of CH100 with different pH values on urediniospore germination

Urediniospore germination of *P. allii* was strongly suppressed in 100-, and 300-fold dilution of CH100 at pH 6.5, 7.7, and 8.5 (Table 1). CH100 was more effective in inhibiting urediniospore germination at high pH than low pH. At pH 8.5 distilled water was also slightly inhibitive to urediniospore germination of the pathogen but not at pH 4.5, 6.5, and 7.7.

Effect of major ingredients in CH100 on urediniospore germination

Germination of *P. allii* urediniospores was significantly (P=0.05) decreased by treatment with CH100, $CaCl_2$, $Ca(NO_3)_2$, CaO, and beef extract, but was not affected by treatment with KNO_3, S-H extract, cabbage extract, and tobacco extract (Table 2). CH100 was more effective in inhibiting urediniospore germination and enhancing lysis of germ tubes than its individual ingredients (Data not shown).

TABLE 3. Effect of population densities of 16 yeast isolates on urediniospore germination of *Puccinia allii* on detached leaves of Chinese leek after 24 hr at 16 C

Yeast isolate[1]	Germination (%)				Coefficient of determination
	Yeast concentration (cfu/ml)				
	0	10^2	10^4	10^6	
Rhodotorula spp.					
YCR-051	47	42	41	47	$R^2=0.31$, $P<0.0821$
YCR-064	45	38	40	32	$R^2=0.38$, $P<0.0452$
YCR-077	42	48	35	39	$R^2=0.15$, $P<0.3433$
YCR-089	50	51	25	15	$R^2=0.86$, $P<0.0001$
YCR-090	48	43	46	43	$R^2=0.10$, $P<0.4912$
YCR-093	42	45	44	28	$R^2=0.76$, $P<0.0001$
YCR-099	50	37	26	17	$R^2=0.94$, $P<0.0001$
Cryptococcus spp.					
YCP-022	52	34	19	6	$R^2=0.92$, $P<0.0001$
YCP-035	46	42	35	35	$R^2=0.36$, $P<0.0552$
YCP-046	38	23	7	14	$R^2=0.85$, $P<0.0001$
YCP-058	46	39	31	9	$R^2=0.91$, $P<0.0001$
YCP-211	48	43	23	0	$R^2=0.96$, $P<0.0001$
YCP-215	44	21	18	15	$R^2=0.88$, $P<0.0001$
YCP-234	41	35	33	40	$R^2=0.45$, $P<0.0233$
YCW-013	38	40	38	36	$R^2=0.18$, $P<0.2754$
YCW-022	45	42	46	36	$R^2=0.31$, $P<0.0842$

[1] Yeast isolates were obtained from leaf surfaces of Chinese leek sprayed with CH100 in the field.

Effect of yeasts on urediniospore germination

Yeast populations, especially *Rhodotorula* spp. and *Cryptococcus* spp. were consistently higher on leaf surfaces of Chinese leek treated with CH100 than on nontreated leaves. Of the sixteen yeast isolates, YCR-051, YCR-064, YCR-077, YCR-087, YCR-090, YCR-093, and YCR-099 were identified as *Rhodotorula* spp. and YCW-013, YCW-022, YCP-022, YCP-035, YCP-046, YCP-058, YCP-211, YCP-215, and YCP-234 were identified as *Cryptococcus* spp.. Isolate YCR-099, *R. glutinis*, was predominant on leaf surfaces of Chinese leek treated with CH100.

Yeast isolates, YCP-022, YCP-046, YCP-058, YCP-211, YCP-215, YCR-089, YCR-093, and YCR-099 at concentrations ranging from 10^2 to 10^6 cfu/ml, significantly ($P<0.0001$) inhibited urediniospore germination of *P. allii* (Table 3). Increased concentrations of yeasts were associated with increased inhibition to the pathogen. Urediniospore germination was negatively and quadratically related to the log transformation of the antagonistic yeast concentrations.

Effect of CH100 and its major ingredients on the growth of yeasts

Proliferation of the yeast *R. glutinis*, isolate YCR-099, on leaves of Chinese leek was stimulated by CH100, $CaCl_2$, and CaO, but was not affected by treatment with $Ca(NO_3)_2$, KNO_3, S-H extract, beef extract, cabbage extract, and tobacco extract (Fig. 2). However, proliferation of *Cryptococcus* sp., isolate YCP-215, was stimulated by treatment with CH100, S-H

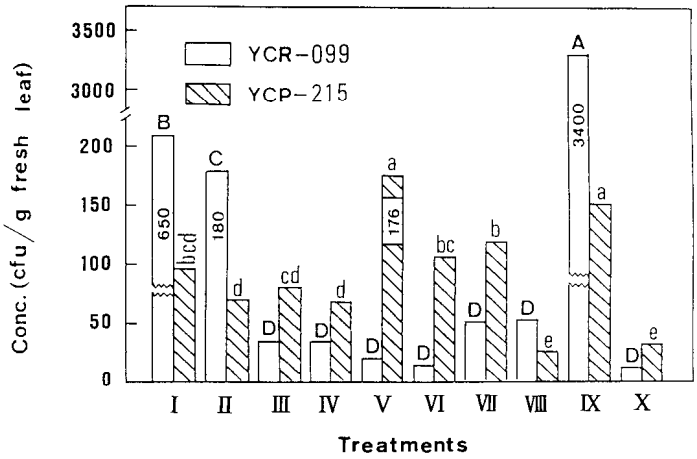


Fig. 2. Effect of CH100 and its major ingredients on the multiplication of *Rhodotorula glutinis* (isolate YCR-099) and *Cryptococcus* sp. (isolate YCP-215) on detached leaves of Chinese leek after 48 hr at 16 C. (I: $CaCl_2$; II: CaO; III:$Ca(NO_3)_2$; IV: KNO_3; V: S-H extract; VI: Beef extract; VIII: Cabbage extract; IX: CH100; X: Distilled water (Control)). Means (n=4) (Columns) for the same yeast isolate with the same letter do not differ significantly ($P=0.05$, Duncan's multiple range test).

TABLE 4. Effect of frequency of spray of CH100 on urediniospore germination and formation of appressoria of *Puccinia allii*

Spraying frequency[1]	Germination (%)	Formation of appressoria (%)	Appressoria on stomata (%)
0	42	16	11
1	33	11	8
2	26	4	2
3	14	2	1
Coefficient of determination[2]	$R^2=0.92$ $P<0.0001$	$R^2=0.90$ $P<0.0001$	$R^2=0.89$ $P<0.0001$

[1] Ten-wk-old plants of Chinese leek were sprayed with CH100 at the rate of 10 ml/L once per week. One week after 3rd-time treatment, 10 plants per replicate were sprayed with urediniospores of the pathogen and covered with polyethylene bags for 24 hr at 16 C. Percentages of spore germination, formation of appressoria and appressoria on stomata were counted under light microscope. Four replicates were conducted per treatment.

[2] Regression equation between urediniospore germination, formation of appressoria, or appressoria on stomata (Y) and spraying frequency (X) was $Y=41.9-6.9X-0.75X^2$, $Y=15.9-6.3X+0.5X^2$, or $Y=11.3-4.9X+0.44X^2$, respectively.

extract, cabbage extract, beef extract, $CaCl_2$, $Ca(NO_3)_2$, CaO, and KNO_3, but not by tobacco extract (Fig. 2). CH100 was more effective in stimulating proliferation of YCR-099 and YCP-215 than its individual ingredients.

Effect of CH100 and a pink yeast on urediniospore germination

Addition of *R. glutinis* (isolate YCR-099) at 10^3 to 10^7 cfu/ml significantly ($P<0.0168$) improved inhibition of urediniospore germination of *P. allii* by a 300-fold dilution of CH100 (Fig. 3). The relationship between urediniospore germination (Y) and log transformation of the yeast cell concentration (X) in 300-fold dilution of CH100 was negatively quadratic regression ($Y=22.3-1.08X+0.03X^2$; $R^2=0.46$, $P<0.0168$). Inhibition of urediniospore germination of the pathogen by 100-fold dilution of CH100 was not significantly changed by addition of *R. glutinis*.

Effect of spraying frequency of CH100 on urediniospore germination

Increasing frequency of treatments with CH100 reduced the percentages of urediniospore germination and appressorial formation (Table 4). Plants treated three times with CH100 showed marked suppression

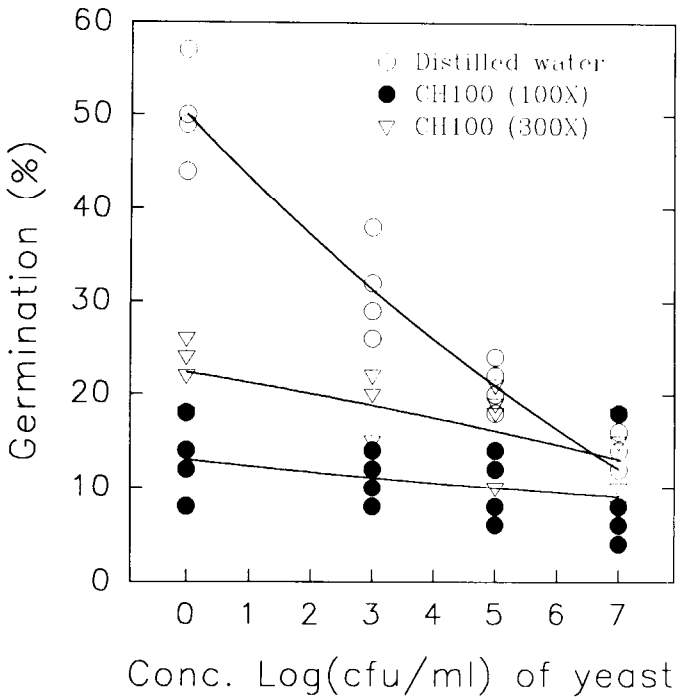


Fig. 3. Effect of CH100 and *Rhodotorula glutinis* (isolate YCR-099) on urediniospore germination of *Puccinia allii* on detached leaves of Chinese leek for 24 hr at 16 C. The relationship between urediniospore germination (Y) and log transformation of the yeast population density (X) in distilled water was $Y=50.0-6.81X+0.20X^2$ ($R^2=0.92$, $P<0.0001$), in 100-fold dilution of CH100 was $Y=13.0-0.71X+0.02X^2$ ($R^2=0.13$, $P<0.3955$) and was $Y=22.3-1.08X+0.03X^2$ ($R^2=0.46$, $P<0.0168$) in 300-fold dilution of CH100.

(28%) of urediniospore germination compared to nontreated plants. Significantly fewer appressoria successfully formed on stomata of leaves treated with CH100 than on those of nontreated plants.

Effect of CH100 on leaf morphology

SEM preparations showed that leaf morphology of Chinese leek treated with CH100 was different from those in non-treated plants. Height of leaf veins in plants treated three times with CH100 was 1.0 μm more taller and width of furrows between leaf vein ridges in 3-time-treated plants was 5.3 μm narrower than ones in non-treated plants (Table 5; Figs. 4, 5). In addition, stomata of treated plants were 0.33 μm shorter than those of nontreated plants, but stoma width was not affected.

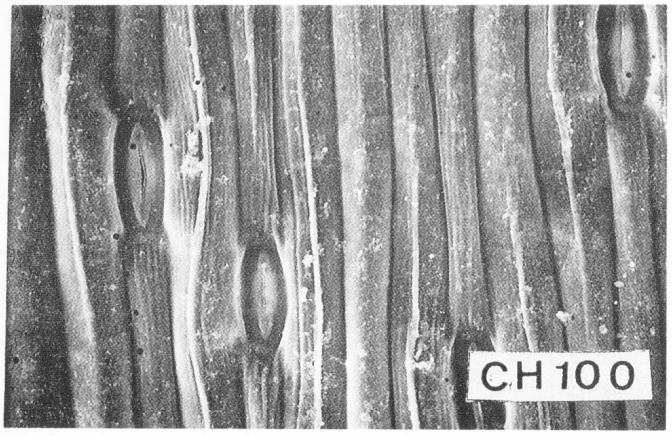
DISCUSSION

CH100, a formulated nutrient, significantly reduced the severity of Chinese leek rust caused by *P. allii*, increased yield of plants, and changed the morphology of leaf surfaces and stomata. CH100

TABLE 5. Effect of frequency of spray of CH100 on morphological structures of leaf veins and stomata of Chinese leek

Spraying frequency[1]	Leaf veins (μm)		Stomata (μm)	
	Ridge height	Furrow width	Length	Width
0	5.40	23.0	1.88	0.63
1	6.96	22.5	1.61	0.63
2	6.81	19.1	1.59	0.65
3	6.40	17.7	1.55	0.66
Coefficient of determination[2]	$R^2=0.26$ $P<0.012$	$R^2=0.35$ $P<0.0023$	$R^2=0.45$ $P<0.0001$	$R^2=0.03$ $P<0.385$

[1] Ten-wk-old plants of Chinese leek were sprayed with CH100 at the rate of 10 ml/L once per week. One week after 3rd-time treatment, the fifth leaves of all plants were sampled and examined by scanning electron microscopy.
[2] Regression equation between height of leaf veins, width of furrows between vein ridges, length of stomata, or width of stomata (Y) and spraying frequency (X) was $Y=5.5+1.6X-0.4X^2$, $Y=23.2-1.2X-0.24X^2$, $Y=1.88-0.23X+0.04X^2$, or $Y=0.64+0.04X-0.01X^2$, respectively.



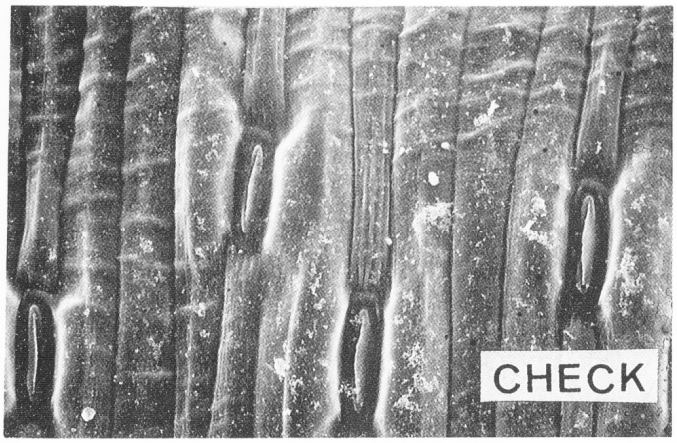
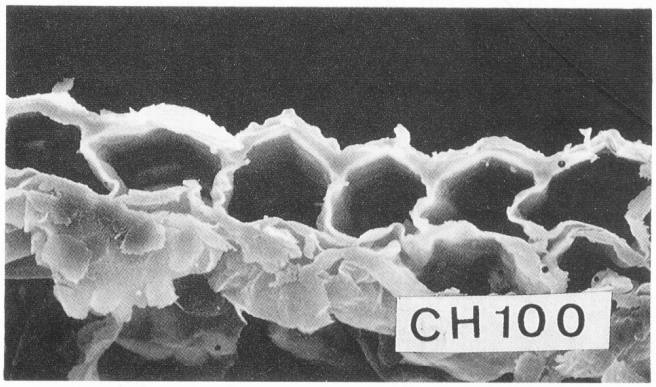


Fig. 4. Scanning electron micrograph showing the morphology of a fifth leaf of Chinese leek sprayed three times with CH100 at the rate of 10 ml/L or distilled water (control) (magnification, 500X). The micrograph indicates that width of furrows between leaf vein ridges in treated plants is narrower than one in non-treated plants.



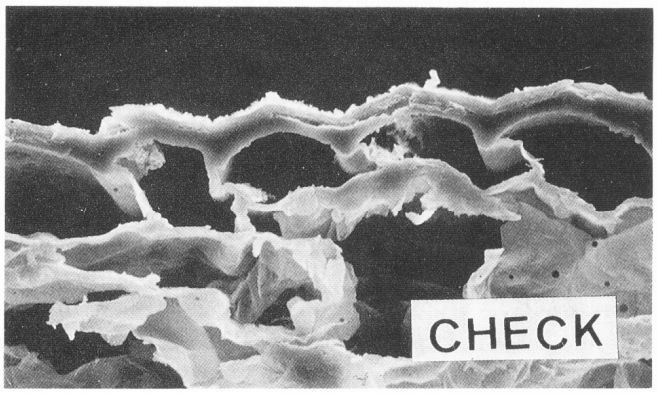


Fig. 5. Scanning electron micrograph showing cross-section morphology of a fifth leaf of Chinese leek sprayed three times with CH100 at the rate of 10 ml/L or distilled water (control) (magnification, 1000X). The micrograph indicates that height of leaf veins in treated plants is taller than one in non-treated plants.

inhibited urediniospore germination of the pathogen, and stimulated proliferation of phyllosphere antagonistic yeasts, *Rhodotorula* spp., and *Cryptococcus* spp.. Calcium chloride and calcium nitrate, the important ingredients of CH100, were responsible for reduction of disease severity and inhibition of urediniospore germination. In addition, since CH100 caused lysis of urediniospores and germ tubes on leaves (J. W. Huang, unpublished), a biological factor apparently plays a role in the CH100 suppression of the pathogen.

The suppressive effect of CH100 on *P. allii* is likely mediated by phyllosphere yeasts. Bashi and Fokkema (2) showed that maximal development of the phyllosphere yeast, *Sporobolomyces roseus* Kluyver & VanNiel, was only achieved in the presence of exogenous nutrients. Huang *et al* (10) showed dramatic increases in yeast populations due to CH100, and this study has shown inhibition of urediniospore germination by several strains of phyllosphere yeasts such as *R. glutinis* (isolate YCR-099) and *Cryptococcus* sp (isolate YCP-215). Although eight of the 16 yeast isolates obtained from leaves treated with CH100 were antagonistic to *P. allii*, the mechanism involved in pathogen suppression by these yeasts remains unknown.

In this study, urediniospore germination and appressorium formation were reduced by treatment with CH100. The morphology of leaf surface and stoma was also changed by treatment with CH100 (Table 5; Figs. 4 & 5). Apparently, CH100 induced changes to chemical and physical features of the leaf surface and thereby, created a negative impact on formation of appressoria of *P. allii* at penetration sites. Both chemical and morphological features of leaf surfaces influence germ tube growth of many rust fungi as well as the development of infection structures (1,8). The leaf surface may provide chemical and physical stimuli that orient the growth of germ tubes of urediniospores toward the leaf stomata (14,22). Chemical stimuli such as CO_2 concentration (23), pH gradients (6), or other compounds (22) may be originated from the stomata. In addition, the formation of an appressorium over a stoma requires an additional stimulus, which may be related to the shape of the guard cells (21). The multiple nutrients of CH100 may also enhance the growth of Chinese leek which in turn may increase the morphological resistance to *P. allii*.

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摘　　要

黃振文．1994．合成植物營養液防治韭菜銹病．植病會刊3:9-17．(台中市　國立中興大學植物病理學系)

噴佈250 ppm的無機鈣鹽、鉀鹽與CH100的三種不同酸鹼值之一百倍稀釋液於韭菜植株，每七天一次，連續三次後，證明噴佈氯化鈣、硝酸鈣與鹼性CH100稀釋液的植株，感染韭菜銹病的百分率較對照未施用者少30%左右。利用 *Puccinia allii*夏孢子在韭菜葉段片表面發芽的生物分析法，研究酸鹼值影響CH100的抑菌效應時，發現CH100的一百倍與三百倍鹼性稀釋液(pH 7.7和8.5)較鹼性蒸餾水多抑制26–33%韭菜銹病菌的夏孢子發芽；惟CH100稀釋液的酸鹼值調降至4.5時，它抑制韭菜銹病菌夏孢子發芽的效應隨即喪失。將組成CH100的主要成分分別與韭菜銹病菌的夏孢子混合後，噴佈於韭菜葉片，在16 C，經24小時，僅氯化鈣、氧化鈣、硝酸鈣及牛肉煎汁等四者可抑制本菌7–19%的夏孢子發芽。此外，CH100可促進 *Rhodotorula* spp.及 *Cryptococcus* spp.等酵母菌在韭菜葉表的增殖，其中 *Rhodotorula* spp.的YCR-089，YCR-093與YCR-099及 *Cryptococcus* spp.的YCP-022，YCP-046，YCP-058，YCP-211與YCP-215等八菌株，在菌量10^2–10^6 cfu/ml時，均具有顯著抑制夏孢子發芽的功效。將韭菜葉表出現頻率最高的 *Rhodotorula glutinis* (YCR-099菌株)與CH100混合使用，證明其有助於提高CH100三百倍稀釋液之抑菌效果。接種韭菜銹病菌的夏孢子在施用過不同次數的CH100之韭菜葉片上，發現夏孢子的發芽率，附著器的形成率與其在氣孔上形成附著器的百分率隨韭菜施用CH100的次數增加而遞減。由掃描電子顯微鏡觀察，發現施用CH100的韭菜，其葉片的葉脈與氣孔形態發生明顯的改變，其中施用CH100者的葉脈比不施用者高挺且脈與脈間距離變窄。此外，施用者的氣孔長度也變得較短。顯然，CH100防治韭菜銹病的機制是由下列三種效應共同表現的結果，即：(1)它含有抑制夏孢子發芽的成分；(2)它可促進韭菜葉表酵母菌 *Rhodotorula* spp.與 *Cryptococcus* spp.等菌的增殖，促使夏孢子的發芽率下降；(3)它提供綜合性的植物營養元素如鈣與鉀等，強壯韭菜植株的發育，並改變葉片的形態，誘使韭菜植體的抗病性增強。

關鍵詞：植物營養液、韭菜銹病、酵母菌、非農藥防治。