Original Articles

Effect of Sodium Bicarbonate on Each Growth Stage of Cucumber Powdery Mildew Fungus (*Sphaerotheca fuliginea*) in Its Life Cycle*

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Sodium bicarbonate (SBC) has a control effect against citrus penicillium decay and several other plant diseases. However, the inhibitory effect varies because of crystallization of the chemical on the plant. A SBC preparation to which an emulsifier used as a food additive was added was considered to be a practical control chemical against powdery mildew of cucumber, egg plant, strawberry, etc. For the purpose of clarifying the characteristic effect of the chemical, a study was made of its effect on cucumber powdery mildew fungus, *Sphaerotheca fuliginea*, at various growth stages and the following results were obtained. 1) Direct spray of SBC at a concentration of 2,000 ppm inhibited 80~100% of conidial germination. 2) In hyphal elongation, there was little difference between this treatment and water-treatment, and no great inhibitory effect was observed. 3) The chemical did not to control conidiophore formation very well, the control percentage being only 38%. 4) The number of conidia formed per conidiophore in about 20 hours was 5 in untreated sections, whereas in the SBC treated sections no conidia were formed. 5) Treatment of new conidia with SBC resulted in the formation of small projections on the surface and rupture of the cell wall under a slight pressure, with subsequent projection of the contents. Such conidia could not infect cucumber plant and the SBC preparation greatly inhibited conidial dispersion.

INTRODUCTION

As stated in our previous reports,^{1,2)} it was found that the effect of sodium bicarbonate (SBC) varied when this chemical was used alone for plant disease control. However, a stabilized result was obtained in the inhibitory effect by adding a surfactant or an emulsifier, used as a food additive, at a rate of 0.1% or 0.01%.³⁾ In order to enhance the safety of the chemical for agricultural use, preparation was made in combination with SBC-glycerine fatty acid ester, the latter being added as an emulsifier. Since the formulation was found to be effective against powdery mildew in vegetables, field tests were made at agricultural

experimental stations in various places over a period of two years to prove its effectiveness.⁴⁾ The results revealed that it was effective if sprayed several times at weekly intervals. From the standpoint of safety, the formulation was judged to be good for practical use.⁵⁾ However, with the view that it would be more effective to establish a control system with a better knowledge of its properties and chemical effects, it was decided to clarify its characteristics on the life cycle of the powdery mildew fungus.^{6,7)}

MATERIALS AND METHODS

1. Inhibition Test of Conidial Germination a. Inhibition of conidial germination by inoculation after spraying SBC formulation

The SBC preparation was diluted at a concentration of 2,000 ppm. The solution was

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sprayed with a spray gun (2 kg/cm²) on cucumber plants at first leaf stage (cultivar: Sagami-hanjiro, about 14 days after seeding) and then wind-dried. To obtain an inoculum on cucumber, powdery mildew fungus was inoculated beforehand with artificial wind on cucumber and cultured for 7~10 days, and clearly formed conidia were used as the inoculum. Inoculation was carried out by making a lesion cut 1 cm×1 cm in size within the area previously marked with a magic marker and touching it on healthy marked area of leaves. The inoculated plant was then grown for 3 days in a common greenhouse (heated to 20°C). A tape adhesive on both sides was stuck on the inoculated portion and held the spores on a tape. Germination was observed under an optical microscope by sticking the tape on a slide glass and the percentage of germination and inhibition rate were calculated.

b. *Inhibition of conidial germination by SBC formulation spray after inoculation of the pathogen*

Cucumber powdery mildew fungus was inoculated on cucumber plants, and when conidia formed on conidiophores (3~4 days after inoculation) these newly formed conidia were inoculated on cucumber plants. After incubation of the material for 24 hours and observation of new conidial formation the chemical was sprayed. The lesion colony was cut into 1 cm×1 cm sections and inoculation was carried out by sticking the section to a healthy cucumber plant. After 24 hours the section was removed with tape adhesive on both sides, and stuck on a slide glass for observation under an optical microscope.

The percentage of germination and inhibitory rate was then calculated.

2. *Inhibition of Hyphal Growth*

Inoculation of conidia of *S. fuliginea* by sticking was carried out on healthy cucumber leaves by the above method. The inoculated material was cultured for 2~3 days in a common greenhouse and the chemical was sprayed onto the material when short hyphae were vaguely observed. During the following 2 days the material was incubated in the greenhouse. On confirmation of further elongation of the hyphae, they were taken off by use of a double

sided-adhesive tape to be stuck on a slide glass. Hyphal elongation in the treated area was compared with that in the untreated area. Classification was made according to the following as an index for hyphal elongation. Hyphae are short and a little longer than a germ tube (no conidiophore formation in a field of view under 150× microscope) (+), hyphae are short and 1~2 conidiophores (++), colonies are formed, but hyphae are scarce and distinguishable (+++), number of hyphae is someway countable (about 20 conidiophores) (++++), and colonies are so thick that separate hyphae are indistinguishable (about 45 conidiophores) (++++++).

3. *Inhibition of Conidiophore Formation*

In the same way, inoculation by sticking was carried out on healthy cucumber leaves, and the inoculated material was cultured in a greenhouse for 2~3 days; after confirmation of conidial germination and their development into hyphae, the chemical was sprayed. The treated material was cultured for 2 days and at the time when conidial formation on the untreated area was observed, each area was stuck in the same way by the method mentioned above, on a double-sided adhesive tape which was then attached to a slide glass and observed under a microscope. Three samples of lesions from each area in the colony were taken to count fresh conidiophores and the percentage of conidiophore formation and inhibitory rate was calculated.

4. *Inhibition of Conidial Formation*

Inoculation of conidia of *S. fuliginea* was carried out on healthy cucumber leaves and the inoculated material was incubated in the greenhouse for 3~4 days. Since formation of a few conidia could be confirmed by observation under an optical microscope, the preparation was sprayed at that time and the material was incubated in the greenhouse for a day (5~6 conidia were formed on an untreated conidiophore).

The mean number of conidia per conidiophore on a total of 10 conidiophores was calculated for the chemically treated and untreated materials, and conidial formation and inhibitory rate were calculated.

5. Effect on Conidial Morphology

Inoculum obtained similarly to the above mentioned method (cucumber lesion colony cut 1 cm × 1 cm) was stuck on healthy cucumber plants for inoculation. The inoculated material was cultured in the greenhouse for the following 4 days and at the time of conidial formation on conidiophores the preparation was sprayed. After spraying, the material was again kept in the greenhouse for one day, and a 5 mm × 5 mm section of the undried and non-vapor fixed sample was stuck on a specimen stub with double-sided adhesive tape. The fine structures of conidia and conidiophores were observed by a MINI SEM model scanning electron microscope.

6. Inhibition of Conidial Dispersion

After inoculation of conidia of *S. fuliginea* by sprinkling, the material was cultured in the greenhouse for 6 days, and when lesion colonies were confirmed, the chemical was sprayed on these affected leaves. In spraying, care was taken that particles of the chemical adhered only to the leaf surface and did not drop from it. Following this treatment the sprayed material was gradually wind-dried and kept in the greenhouse for about 24 hours. The lesion colonies were cut into 1 cm × 1 cm sections and with each section being held about 3 cm above a slide glass, the leaf surface was patted 20 times with the tip of a finger to accelerate conidial dispersion. The conidia attached on the slide glass were counted under a microscope to compare the degree of detachment with that of untreated sections.

7. Pathogenicity of Conidia Treated with the Chemical

On the other hand, inoculation by sprinkling was carried out on a marked location of healthy

cucumber plants using the same method as above (6). The inoculated cucumber plants were kept in a greenhouse for 18 days and observed until lesions had formed. The degree of lesion formation was classified as follows: lesion colonies formed with a considerably rapid rate of development (++), lesion colonies formed to a degree that they could be recognized with difficulty (+), no lesions formed (—). The number of lesions per true leaf was also counted for comparison with that of the untreated area, and the average number of lesions and the control value were calculated.

8. Effect of the Chemical on the Development of Lesions

About 7 days after inoculation by sprinkling of cucumber plants (at first leaf stage) with fresh conidia, the number of lesions was counted. Immediately after counting the number of lesions the chemical was sprayed on 2 plants in each section, and the material was cultured in a greenhouse. On the 7th and 14th days the number of lesions was counted.

RESULTS

1. Inhibition Test of Conidial Germination

a. Inhibition of conidial germination by inoculation after spraying SBC formulation

Cucumber plants were treated with the SBC preparation at a concentration of 2,000 ppm at the first leaf stage.

After wind-drying of the material, it was inoculated with newly formed conidia, and at 24 and 48 hours after inoculation the percentage of germination was compared with that of the untreated section. As shown in Table 1, the result revealed no appreciable difference between untreated and treated material. This is believed to be because of the infrequent of direct contact of SBC with conidia.

Table 1 Effect of sodium bicarbonate (SBC) on the conidial germination of *Sphaerotheca fuliginea* on pre-treated cucumber leaf.

Chemical	Concentration (ppm)	Conidial germination (%)		Inhibition (%)
		24 hr	48 hr	48 hr
SBC + GFE	2,000	37.3	55.8	10
Water	0	38.5	62.0	—

SBC: Sodium bicarbonate, GFE: 0.1% glycerine fatty acid ester.

Table 2 Effect of sodium bicarbonate on the conidial germination of *Sphaerotheca fuliginea*.

Chemical ^{a)}	Concentration (ppm)	Conidial germination ^{b)} (%)	Inhibition (%)
SBC+GFE	2,000	0	100
SBC	2,000	2.9	95
Water	0	44.4	23
Check	—	57.0	0

^{a)} Chemicals were sprayed on conidia on cucumber leaves.

^{b)} Newly formed conidia were used as inocula.

GFE: 0.1% glycerine fatty acid ester.

b. Inhibition of conidial germination by SBC formulation spray after inoculation of the pathogen

The percentage of germination on untreated material was 60%, while germination was slightly inhibited in the water-treated section. However, in the case of treatment with SBC alone, germination was inhibited to a considerable extent.

In the SBC-glycerine fatty acid ester (GFE) treated section, germination was inhibited 100% (Table 2).

2. Inhibition of Hyphal Growth

Inoculation of conidia of the pathogen on cucumber leaves was done; 5 days after inoculation the hyphae were treated with SBC-GFE and thereafter their growth was observed. When compared with the hyphal condition observed shortly before chemical treatment, the growth 3 days after treatment was considerable.

Table 3 Effect of sodium bicarbonate on the mycelial growth of *Sphaerotheca fuliginea*.

Chemical ^{a)}	Concentration (ppm)	Degree of mycelial growth ^{b)}
SBC+GFE	2,000	+++
SBC	2,000	++
Water	0	++++
Check	—	+++++
Mycelia at the time of chemical treatment		+

^{a)} Chemical treatment was done 5 days after inoculation.

^{b)} Observation was made 8 days after chemical treatment.

GFE: 0.1% glycerine fatty acid ester.

The water-treated section had slightly less hyphal growth than the untreated section, and in the section treated with SBC alone 8 days after treatment, the growth was approximately less than one-half. Growth in the SBC-GFE section was slightly better than that in the section treated with SBC alone. This reveals clearly that neither SBC nor SBC-GFE is very inhibitive against hyphal growth (Table 3).

3. Inhibition of Conidiophore Formation

Since conidiophore formation starts about 5 days after conidial inoculation, the chemical was sprayed at that time. Three days later the degree of conidiophore formation was observed under a microscope. Based on the result obtained, the percentage of inhibition of formation was calculated and compared with that of the untreated section. In the water-treated section, the percentage of inhibition of conidiophore formation was 60%, while in the section treated with SBC alone, about 96% was inhibited.

On the other hand, in the SBC-GFE section, inhibition rate was only 38%, less than both the section treated with SBC alone and the water-treated section (Table 4).

4. Inhibition of Conidial Formation

Since newly formed conidia were observed about 7 days after conidial inoculation on cucumber leaves, the chemical was sprayed after dropping of the newly formed conidia from conidiophores.

Twenty hours after spraying, the number of newly conidia was counted to compare with that of the untreated section. The number of conidia formed on a conidiophore was 5~6 in the untreated section. Although water treat-

Table 4 Effect of sodium bicarbonate on the formation of conidiophores of *Sphaerotheca fuliginea*.

Chemical ^{a)}	Concentration (ppm)	Number of newly formed conidiophores ^{b)}	Percentage of inhibition (%)
SBC+GFE	2,000	84	38
SBC	2,000	5	96.3
Water	0	54	60.0
Check	—	135	0

^{a)} Chemical treatment was done 5 days after inoculation.

^{b)} Observation was made 8 days after chemical treatment.

The number of newly formed conidiophores was measured as the total number per 3 field of view under 150× microscope.

GFE: 0.1% glycerine fatty acid ester.

Table 5 Effect of sodium bicarbonate on the conidial formation of *Sphaerotheca fuliginea*.

Chemical ^{a)}	Concentration (ppm)	Number of conidia formed on conidiophore ^{b)}	Percentage of inhibition (%)
SBC+GFE	2,000	0	100
SBC	2,000	1.08	79.1
Water	0	4.31	16.6
Check	—	5.17	0

^{a)} Conidiophores pulled off old conidia were treated with chemicals.

^{b)} Newly formed conidia were measured 20 hours after chemical treatment.

GFE: 0.1% glycerine fatty acid ester.

Table 6 Effect of sodium bicarbonate on the conidial morphology of *Sphaerotheca fuliginea*.

Chemical ^{a)}	Concentration (ppm)	Number of conidiophores with normal conidia ^{b)}	Percentage of conidiophores with abnormal conidia (%)
SBC+GFE	2,000	24	74
SBC	2,000	45	52
Water	0	80	15
Check	—	94	0

^{a)} Newly formed conidia were treated with chemicals.

^{b)} The number of conidiophores with normal conidia was measured as total number per 11 field of view under 150× microscope.

GFE: 0.1% glycerine fatty acid ester.

ment retarded conidial formation, only a little compared with non-treatment, in the SBC and SBC-GFE sections, conidial formation was inhibited to a considerable extent, the percentage of inhibition being 80% and 100%, respectively (Table 5).

5. Effect on Conidial Morphology

About 7 days after conidial inoculation on the surface of cucumber leaves, conidia were

formed. The effect of SBC on these newly formed conidia was observed. Distinction between normal and abnormal conidia was made under a microscope and the number of conidiophores of each type was counted. Based on the conidiophore number in the untreated sections (Fig. 1-1), those having abnormal conidia and the number of normal conidia of the treated section were counted. The percentage of conidiophores bearing abnormal

Fig. 1 Effect of sodium bicarbonate and its formulation on the conidial morphology.

- 1 Newly formed conidia and conidiophores (Check). c: conidium, cp: conidiophore, m: mycelium.
- 2 Water treated conidia and conidiophores. Conidia were released from the tips of conidiophores by the pressure of water spray, but conidia were not deformed by the treatment. c: conidia, cp: conidiophore, m: mycelium.
- 3 SBC solution (2,000 ppm) treated conidia and conidiophores. Many conidia were burst by the treatment. c: conidium, cp: conidiophore, m: mycelium.
- 4 SBC formulation (2,000 ppm) treated conidia and conidiophores. Conidia were not burst, but were deformed by the treatment and unidentified small projections appeared on their surface. c: conidium, cp: conidiophore, m: mycelium.

conidia was 15% in the water-treated section (Fig. 1-2), 52% in the SBC section (Fig. 1-3), and 74% in the SBC-GFE section (Fig. 1-4). It was found that SBC induced abnormal conidia even after a formation of conidia (burst (Fig. 1-3) and abnormal projections on conidial surfaces (Fig. 1-4)) (Table 6).

6. Inhibition of Conidial Dispersion

The effect of treatment with SBC preparations containing various emulsifiers as additives on conidial dispersion was studied;

the results are shown in Table 7. The effect on dispersion in water-treated sections was similar to that in the untreated sections. By treating with SBC, conidial dispersion was accelerated by 161%. However, in the sections treated with SBC-soybean lecithin (SL), SBC-sucrose fatty acid ester (SE), or SBC-GFE, dispersion was inhibited to the extent of 78, 82 and 100% respectively, and it was shown that addition of an emulsifier caused a further remarkable inhibition of conidial dispersion.

Table 7 Number of conidia dispersed from chemically treated lesions on slide glass.

Chemical	Concentration (ppm)	Number of conidia ^{a)}	Percentage of conidia (%)
SBC+SL	2,000	19	21.6
SBC+SE	2,000	16	18.2
SBC+GFE	2,000	0	0
SBC	2,000	230	261.0
Water	0	87	98.9
Check	—	88	100

^{a)} Conidia were dropped from a lesion (1 cm × 1 cm) treated with chemicals.

SL: 0.1% soybean lecithin, SE: 0.1% sucrose fatty acid ester, GFE: 0.1% glycerine fatty acid ester.

Table 8 The pathogenicity of conidia treated with various sodium bicarbonate formulations.

Chemical	Concentration (ppm)	Number of disease lesions ^{a)} per leaf	Disease symptom ^{b)}
SBC+SL	2,000	2.0	
SBC+SE	2,000	1.5	
SBC+GFE	2,000	0	—
SBC	2,000	2.5	+
Water	0	4.0	+
Check	—	4.5	++

^{a)} Conidia were dropped from a lesion (1 cm × 1 cm) treated with chemicals.

^{b)} Observation was made 9 days after inoculation.

SL: 0.1% soybean lecithin, SE: 0.1% sucrose fatty acid ester, GFE: 0.1% glycerine fatty acid ester.

7. Pathogenicity of Conidia Treated with the Chemical

Inoculation of the pathogen conidia was carried out on cucumber true leaves. After observation of lesion colonies, the chemical was sprayed on them. A lesion of each section sprayed with the chemical was used as the inoculum and inoculated on to cucumber plants. The number of lesions formed after 7 days was calculated. In this experiment no conspicuous difference was observed between the untreated and the water-treated section. However, in the SBC treated section, the number was reduced to about one-half, and in the sections treated with SBC–SL, SBC–SE or SBC–GFE, a remarkable decrease in the number of lesions was observed (Table 8).

In spite of a large number of conidial dispersions on the slide glass, the ability to form lesions on the SBC section was low, which suggested that SBC weakens conidial pathogenicity (Table 8).

8. Effect of the Chemical on the Development of Lesions

Inoculation of conidia on healthy cucumber plants was carried out, and 6 days later the number of lesions was counted and the materials was treated with the chemical. Following incubation of the treated material for 18 days, the increase or decrease in the number of lesions of each section was counted (Table 9). In the untreated section, the lesions increased by as many as 40 in number up to the time of counting, and in the water-treated and SBC–SL sections, they increased by 60% and 71% respectively. The SBC–SE and SBC sections also had a respective increase of 20% and 14% in the number of lesions. On the other hand, in the SBC–GFE section a reduction of about 10% was observed. This suggested that the inhibitory effect of SBC on the increased of lesions varied to some extent with the emulsifier added in the preparation. From these data, the mode of action of SBC against the life cycle of *Sphaerotheca fuliginea* is summarized in Fig. 2. Direct spray of the SBC formulation led to the strong inhibition of conidial

Table 9 Effect of sodium bicarbonate treatment after symptom occurrence on disease development.

Chemical	Concentration (ppm)	Number of lesions before treatment ^{a)}	Increased Number of lesions after treatment ^{b)}	Percentage of increased lesions after treatment (%)
SBC+SL	2,000	35.5	25.5	+71
SBC+SE	2,000	45.0	9.0	+20
SBC+GFE	2,000	26.8	-2.5	-10
SBC	2,000	39.0	5.5	+14
Water	0	34.3	22.5	+60
Check	—	36.0	40.8	+113

^{a)} 17 days after inoculation.^{b)} 20 days after treatment.

SL: 0.1% soybean lecithin, SE: 0.1% sucrose fatty acid ester, GFE: glycerine fatty acid ester.

Fig. 2 The diagram of growth stage of *Sphaerotheca fuliginea* and the inhibitory site of SBC formulation.

Arrow indicates sodium bicarbonate formulation (SBC) treatment.

1. Normal, 2. SBC treatment before inoculation, 3. SBC treatment after inoculation, 4. SBC treatment at hyphal elongation, 5. SBC treatment at the emergence of conidiophore, 6. SBC treatment at conidial formation, 7. Inoculation of SBC treated conidia.

germination (2-arrow). Then, conidial formation and the dispersion of conidia were inhibited (5, 6-arrow), and in addition, the formation of conidiophores was also inhibited to some extent (4-arrow).

DISCUSSION

SBC is effective on various plant diseases, especially powdery mildews of vegetables.⁷⁾ However, its preventive effect varied when sprayed in an aqueous solution, because of its crystallization on the plant. In an effort to minimize the instability of the effect, a preparation was made with glycerine fatty acid ester, sucrose fatty acid ester, and soybean lecithin, which were chosen from various

emulsifiers. Using a sample chemical prepared with SBC-glycerine fatty acid ester (GFE), which had shown a stabilized effect against powdery mildew of vegetables and was therefore chosen from the above emulsifiers, the effect was studied.³⁾

Cucumber powdery mildew (*Sphaerotheca fuliginea*), was separately treated with SBC and SBC-GFE at concentrations of 2,000 ppm, during principal growth stages of the life cycle of the fungus, including conidial germination, hyphal elongation, conidiophore formation, conidial formation and conidial dispersion. The effects of the chemicals on fungal growth, conidial morphology and pathogenicity were studied. The inhibitory potency of SBC is

shown by the fact that the chemical inhibits conidial germination very well when conidia are treated directly.⁶⁾ In addition, it remarkably inhibits conidial formation brought about following conidiophore formation. It was also proved that SBC influenced conidial morphology so much that it caused morphologic anomaly. Such conidia were inoculated on cucumber plants and their pathogenicities were investigated. The result showed a remarkable decline in pathogenicity and, accordingly, a reduction in incidence. It is believed that the inhibitory effect of SBC is particularly strong at the stage between conidial formation and germination of the fungus. Such characteristics of SBC were shown even more strikingly with a sample prepared by adding glycerine fatty acid ester to SBC at a rate of 0.1%; this greatly inhibited conidial germination and formation,⁶⁾ and also caused morphologic anomaly of conidia. It was found that these effects were far superior to those of SBC alone. The pathogenicity of SBC-GFE treated conidia was found to be remarkably reduced when used as inoculum. Further, in spraying the preparation on lesions already formed, the number of lesions remained unchanged for a week or two and an occasional decrease (treatment effect) was even observed. Although this phenomenon is considered due to inhibition of conidial formation and conidial germination of SBC-GFE, the dispersion control effect which cannot be observed with SBC alone may be a major contributing factor. However, inhibition of conidiophore formation, as well as controlled hyphal elongation which was observed with SBC alone, was somewhat reduced with SBC-GFE. Although the following explanation for the cause of the above is mere conjecture, an SBC preparation has a lowered surface tension, which greatly accelerates the effect of wind-drying after spraying and this may have the effect of minimizing damage against hyphal and conidiophore formation.

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要 約

キュウリうどんこ病菌 (*Sphaerotheca fuliginea*) の生育過程に及ぼす炭酸水素ナトリウムの影響*

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炭酸水素ナトリウム (SBC) はカンキツ緑かび病をはじめ数種の植物病害に抑制効果を有するが、植物体上で結晶化し、効果にふれがある。食添用乳化剤を加えて製剤化した結果、キュウリ、ナス、イチゴなどのうどんこ病防除薬剤として実用性のあることが判定された。本剤の作用特性を知る目的で、キュウリうどんこ病菌 *Sphaerotheca fuliginea* の各生育過程に及ぼす影響を検討し、次のような結果が得られた。1) 直接 SBC 剤 2,000 ppm を散布することによって、分生孢子発芽の 80~100% を阻害した。2) 菌糸伸長には水処理と大差はなく、強い阻害効果は認められない。3) 分生子梗形成の抑制率は 38% であって、あまり抑制的ではなかった。4) 分生孢子形成は約 20 時間で無処理 1 分生子梗あたり 5 個であったが、SBC 剤処理区ではまったく形成されなかった。5) 新生分生孢子に SBC 剤を処理したところ、表面に小突起ができ、わずかな圧力で細胞壁が破れ、内容物がとび出した。このような分生孢子を接種しても発病にいたらず、そのうえ、SBC 剤は分生孢子的飛散を著しく抑制した。

* 炭酸水素ナトリウムの植物病害防除に関する研究 (第2報)