イチゴうどんこ病に対する薬剤の効果を簡易かつ確実に評価できる試験法

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A Simple and Reliable Method for Evaluating the Effectiveness of Fungicides for Control of Powdery Mildew (*Sphaerotheca macularis*) on Strawberry

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Abstract

A simple and reliable method was established for evaluating fungicide efficacy in the control of strawberry powdery mildew. The lower surface of newly developed leaflets of strawberry runner-tip plantlets was the best for assay inoculation, and disease severity could be visually evaluated with ease 7 days after inoculation. The youngest leaflets were more susceptible than the intermediate leaflets, and the oldest leaflets were hardly infected by the fungus. The colonies on the inoculated lower surface of runner-tip plantlets were observed clearly 7 days after inoculation and remained alive for 28 days. Disease severity was consistently high when sprayed with conidial suspension containing more than 5.0×10^4 conidia/ml. Maximum infection of inoculated leaflets was observed when incubated at near 20°C. Infection frequency at 15°C was greater than at 25°C. No disease development was observed at or above 30°C. When leaflets were sprayed with effective fungicides before inoculation, disease development was remarkably suppressed. The effects of fungicide were more clearly evaluated with leaflets in test tubes than those on whole plants in the vinyl-covered house. These results indicate the validity of this method for the accurate evaluating of fungicide effectiveness. This method will be adapted to the screening of fungicides for powdery mildew control.

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Key words : strawberry powdery mildew, method for evaluation, fungicide efficacy.

INTRODUCTION

Since a new cultivar Toyonoka of strawberry (*Fragariae × ananassa* Duch) was brought into a large acreage of cultivation in the Mid-1980s, powdery mildew [*Sphaerotheca macularis* (Wallroth et Fries) Lind] has been a serious problem in strawberry production. Although various fungicides have been used for the control of this disease, they were, in most cases, not effective enough. Mainly DBEDC {complex of bis (ethylenediamine)copper-bis- (dodecylbenzenesulfonic acid} and sterol demethylation inhibitors (DMIs: bitertanol, fenarimol, triforine, triflumizole, myclobutanil) have been sprayed in nurseries and in vinyl-covered houses. However, the incidence of the disease did not decrease in spite of the improvement of spray method because the effectiveness of fungicides is influenced by many factors, especially by the extent of fungicide deposit on the lower surface of leaves in the field[7]. More importantly, poor fungicide efficacy also has been correlated with development of resistance in the fungus to the fungicide[1,3]. Thus a reliable method for assessing these correlations is urgently needed.

This study establishes a reliable method for rapidly evaluating fungicide efficacy in the control of strawberry powdery mildew.

MATERIALS AND METHODS

Plants. Strawberry runner plants, cv. Toyonoka, were used for the experiments. To establish the most reliable inoculation method, lower surfaces of newly developed leaflets of runner-tip plantlets, potted plants, detached young leaflets, and leaf discs (6 mm in diameter) were inoculated with the fungus. Youngest, intermediate, and oldest leaflets of runner-tip and runner plants were used to test the effect of leaf age on infection. Runner-tip plantlets were grown in a vinyl-covered house.

Preparation of inoculum. Conidia of *S. macularis* were collected from several diseased strawberry leaves and fruits in fields at the Nara Prefectural Agricultural Experiment Station. Sporulating colonies

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on leaves were put into a glass vial containing distilled water and condia were detached by brushing. Leaves were inoculated by spraying the conidial suspension onto leaves and leaf discs, or dipping leaves into a conidial suspension, or by rubbing colonies with conidia onto leaves. Leaflets, leaf discs, and plants were inoculated with 1 ml, 1 ml (approximately 3×10^5 spores/cm^2), and 10 ml, respectively, of a conidial suspension adjusted to 10^5 conidia/ml.

Incubation. Inoculated leaflets of runner-tip plantlets, leaf discs or whole plants were incubated at 20°C with a photoperiod of 12 hr under fluorescent light (3000 lux) per day for 1-5 weeks. Inoculated leaflets on runner-tip plantlets were placed into a test tube containing 10 ml distilled water, and were used to investigate the relationship between inoculum density and the development of powdery mildew. Artificially and naturally infected leaflets of runner-tip plantlets were used to study the disease development at 15, 20, 25, and 30°C.

Chemical treatment. Polyoxins (10% WP polyoxin), Baycoral (25% WP, bitertanol), Trifmine (30% WP triflumizole), Rubigan (12% fenarimol), Rally (10% WP myclobutanil), Sanyol {20% EW DBEDC= complex of bis(ethylenediamine)copper-bis-(dodecylbenzenesulfonic acid} and spreader (dialkyldimethylammo nium polynaphthylmethanesulfonate • polyoxyethylene fatty acid ester) were tested for their fungicidal activities. Mildew-free leaflets of runner-tip plantlets were dipped in solutions containing these chemicals at three different concentrations within the range of registration, allowed to dry and then spray inoculated with conidia.

Leaflets and foliar spray tests. Fungicidal effects of bitertanol (100 mg/l) and DBEDC (400 mg/l) assessed with leaflets of runner-tip plantlets in test tubes were compared with those on whole plants in the field. Leaflets of runner-tip plantlets were dipped once into a fungicide before inoculation. Strawberry plants in the field were sprayed with these solutions three times at an interval of a week.

The percentage of leaflet area or leaf-disc surface covered with powdery mildew was assessed 7 days after inoculation, and mildew development was rated, using the following scale : A, more than 50% areas of leaflet or leaf-disc surface were covered by powdery mildew ; B, 25–50% of leaflet covered ; C, two colonies to 25% of leaflet covered ; D, one small colony present ; E, no visible mildew development.

Table 1. Susceptibility of strawberry leaves at different ages to powdery mildew fungus (*Sphaerotheca macularis*)

Inoculation materials	Leaf age	Leaflets diseased (%)	Disease severity[a)]	Days after inoculation[b)]
Leaflet of runner-tip plantlet	undeveloped	8.3	5.2	28
	youngest	82.5	70.8	28
	intermediate	57.5	63.5	28
	oldest	33.3	23.9	28
Rooted plant	undeveloped	0.0	0.0	>30
	youngest	73.3	46.7	>30
	intermediate	13.3	6.7	>30
	oldest	13.3	5.0	>30
Leaf	youngest	28.1	10.0	15
Leaf disc	youngest	30.0	20.0	20

a) Disease severity of each leaflet or leaf discs was assigned to 5 categories based on the following scale ; A : more than 50% on the surface of area of the leaflet infected, B : 25-50% of leaflet, C : two colonies covering covered 25% of leaflet, D : one small mycelial colony, E : no visible mildew development. Disease severity was calculated as follows: $[(4n_A+3n_B+2n_C+n_D)/4\ (n_A+n_B+n_C+n_D+n_E)]\times100$. n_A to n_E represents the number of the reaflets or leaf discs in each category.

b) The days that fungus colony remained alive. Germination of the conidia was observed under the microscope.

RESULTS

Susceptibility of tissues and concentration of inoculum

The lower surface of newly developed leaflets of strawberry runner-tip plantlets was found the best for inoculation, although mycelial colonies appeared on whole plants. The maximum infection and conidial proliferation were observed on the lower surface of runner-tip leaflets. The disease development on leaflets of runner-tip plantlets was consistently greater than those on detached leaflets and leaf discs. Moreover, the youngest leaflets were more susceptible than the intermediate or undeveloped leaflets. The oldest leaflets were hardly infected by the fungus. Mycelial colonies were observed obviously 7 days after inoculation on the lower surface of runner-tip plantlets, and after 9 days on nursery plants. These colonies remained alive for 15

Table 2. Development of powdery mildew on strawberry leaflets of runner-tip plantlets as affected by inoculum density of the pathogen

Conidial[a)] density	Leaflets diseased (%)		Disease severity	
	7[b)]	16	7	16
4.0×10^5	86.7 a[c)]	93.3 a	75.8 a	83.3 a
2.0×10^5	83.3 ab	100.0 a	71.7 a	88.3 a
10^5	63.3 bc	86.7 ab	41.7 b	69.2 ab
5.0×10^4	63.3 bc	93.3 ab	42.5 b	76.7 ab
2.5×10^4	43.3 cd	80.0 b	21.7 bc	57.5 b
1.3×10^4	20.0 de	46.7 c	9.2 c	30.0 c
6.3×10^3	3.3 e	20.0 cd	1.7 c	10.8 cd
3.1×10^3	10.0 e	36.7 cd	3.3 c	20.0 cd
1.6×10^3	0.3 e	6.7 d	0.3 c	5.8 d
0.0	0.0 e	0.0 d	0.0 c	0.0 d

a) Runner-tip plantlets were inoculated with 1 ml of a conidial suspension.

b) Days after inoculation.

c) The same letter after numbers represents no significant difference in Duncan's multiple range test at $p=0.05$ level.

days on detached leaves, 20 days on leaf discs and 28 days on leaflets of runner-tip plantlets (Table 1).

The newly developed leaflets were consistently and successfully infected when inoculated with conidia at a concentration of 1.6×10^3–4.0×10^5/ml. Disease development was more severe as the concentration of conidia was increased. Disease severity was uniformly high over the range of inoculum concentration from 5.0×10^4 conidia/ml to 4.0×10^5 conidia/ml and was low below 1.6×10^3 conidia/ml (Table 2).

Temperatures and inoculation method

Maximum infection of both artificially inoculated and naturally infected leaflets was observed when incubated at near 20°C. Infection frequency at 15°C was consistently greater than at 25°C. No disease development was observed on leaflets incubated at or above 30°C (Table 3).

No differences in effectiveness between spraying conidial suspension and direct application of powdery mildew colonies on leaves to leaflets was observed (data are not shown.).

Table 3. Effects of temperature on powdery mildew development on strawberry leaflets of runner-tip plantlets

Temperature (°C)	Artificially infected leaflets		Naturally infected leaflets	
	Leaflets diseased (%)	Disease severity	Leaflets diseased (%)	Diseased severity
15	90.0	67.8 ab[a]	93.3	57.5 a
20	90.0	82.5 a	86.7	65.0 a
25	63.3	45.0 b	53.3	47.5 a
30	0.0	0.0 c	0.0	0.0 b

a) Numbers in columns followed by the same letter are not significantly different in Duncan's multiple range test at $p=0.05$ level.

Effects of fungicide on leaflets with runner-tip plantlets in test tubes

Strawberry leaflets sprayed with myclobutanil or fenarimol before inoculation were not infected by the fungus. Bitertanol or triflumizole were less effective and polyoxin was ineffective at every dilution. DBEDC without the spreader was utterly ineffective at any concentration. However its effectiveness was increased when the spreader was combined with DBEDC (Table 4).

Comparison of fungicidal effects on leaflets of runner-tip plantlets in test tube and on plants in the vinyl-covered house

The development of strawberry powdery mildew did not occur when bitertanol was applied to leaflets of

Table 5. Fungicidal effects of bitertanol and DBEDC on strawberry powdery mildew as assessed with detached runner leaflets and whole plants in a vinyl-covered house for comparison

Fungicide	Plants[a]			Runner-tip plantlet[b]	
	Diseased leaflets (%)	Disease severity	Diseased fruits (%)	Diseased leaflets (%)	Disease severity
Bitertanol	21.2 b[c]	7.4 b	46.8 ab	0.0 b	0.0 b
DBEDC	12.2 b	4.6 b	14.8 b	10.0 b	2.5 b
Water	60.8 a	30.3 a	63.6 a	53.3 a	23.3 a

a) Bitertanol (100 mg/l) and DBEDC (400 mg/l) were sprayed on plants (100 ml/plant) three times every week (May 6,13,20). DBEDC means the complex of bis (ethylenediamine)copper-bis-(dodecylbenzenesulfonic acid).

b) Leaflets sprayed with fungicide were dried up, and then inoculated by spraying with conidial suspension of 10^5 conidia/ml.

c) Numbers in columns followed by the same letter are not significantly different in Duncan's multiple range test at $p=0.05$ level.

Table 4. Fungicidal effects on strawberry powdery mildew assessed with leaflets of runner-tip plantlets

Fungicide	Low conc.[a]		Intermediate conc.		High conc.	
	Leaflets diseased (%)	Disease severity	Leaflets diseased (%)	Disease severity	Leaflets diseased (%)	Disease severity
Myclobutanil	0.0	0.0 d[c]	0.0	0.0 d	0.0	0.0 d
Fenarimol	0.0	0.0 d	0.0	0.0 d	0.0	0.0 d
Bitertanol	0.0	0.0 d	6.7	2.5 d	0.0	0.0 d
Triflumizole	6.7	2.5 d	0.0	0.0 d	0.0	0.0 d
Polyoxin	13.3	5.0 d	20.0	12.5 cd	13.3	2.5 d
DBEDC	80.0	42.5 ab	53.3	37.5 ab	40.0	27.5 bc
DBEDC+spreader[b]	46.7	7.5 cd	20.0	27.5 b	0.0	0.0 d
Water	73.3	52.5 a	—[d]	—	—	—

a) Diluted with water ; myclobutanil : 12.5 (low), 25 (intermediate), 50 mg (high)/l, fenarimol : 15 (low), 30 (intermediate), 60 mg (high)/l, bitertanol : 50 (low), 100 (intermediate), 200 mg (high)/l, triflumizole : 50 (low), 100 (intermediate), 200 mg (high)/l, polyoxin : 50 (low), 100 (intermediate), 200 mg (high)/l, DBEDC means the complex of bis (ethylenediamine) copper-bis-(dodecylbenzenesulfonic acid) : 100 (low), 200 (intermediate), 400 ml (high)/l.

b) Dialkyldimethylammonium polynaphthylmethanesulfonate•polyoxyethylene fatty acid ester 1 ml/l.

c) Numbers in columns followed by the same letter are not significantly different Duncan's multiple range test at $p=0.05$ level.

d) Not tested.

runner-tip plantlets in test tubes before inoculation. DBEDC was slightly less effective than bitertanol. When whole plants in the vinyl-covered house were sprayed with these two fungicides three times after the occurrence of the disease, subsequent disease development on leaves was suppressed. This was also observed with disease development on detached leaflets of runner-tip plantlets in test tubes. DBEDC was found more effective than bitertanol in the field test (Table 5).

DISCUSSION

In spite of extensive application of fungicides, the disease has not been controlled effectively to the extent that satisfy farmers. The low control efficiency of fungicides may be in part due to the appearance of resistant strains[1], but more importantly to the insufficient fungicide deposit on lower surface of leaves[7].

To clearly distinguish fungal resistance to fungicides from other factors, the development of appropriate procedure for assaying fungicidal efficacy to powdery mildew of strawberry was a necessity. Monitoring methods for detecting increased resistance in powdery mildew of barley and cucumber have been developed and its decreasing sensitivity to systemic fungicides has been investigated[3-6]. Although the efficacy of fungicides for controlling powdery mildew of strawberry has been examined in the field, an easy and reliable monitoring method had not been developed. The method described here is simple and reliable for evaluating fungicidal effects.

We observed that powdery mildew of strawberry could be produced on newly developed leaflets of detached runner-tip plantlets in the laboratory, hence these leaflets were thought to be the best for the assay of fungicidal activities. These leaflets were uniformly infected by the fungus when inoculated with conidial suspensions at concentrations greater than 5×10^4/ml. Disease development occurred in 1 week and disease severity could be visually evaluated with ease. Runner-tip plantlets with leaflets could be collected at any time from nurseries and vinyl-covered house.

Disease development occurred at temperatures in the range of 15°C to 25°C, maximum development occurring at 20°C, but was completely suppressed at 30°C. Disease severity was greater at 15°C than at 25°C. These results on the effect of temperature on disease development agree with those previously reported[2,8]. Thus, the method we established can be utilized not only for the assay of fungicidal efficacy but also for determining effects of various environmental factors affecting disease development.

When leaflets of runner-tip plantlets were sprayed with fungicides before the infection, disease development was remarkably suppressed and the fungicide effectiveness was evaluated more accurately and more rapidly on detached leaflets in test tubes than on whole plants in the field. Insufficient field performance of bitertanol might be caused by spraying after disease development or by the presence of resistant strains in the field.

Assays of sensitivity of the cucumber powdery mildew fungus indicated a decreased sensitivity to DMIs[3,4]. The development of fungicide resistance occurs commonly in the chemical control of cucumber powdery mildew. Since both leaves and fruits are infected by the fungus in strawberry, the loss in strawberry production is much greater than in cucumber. However, the fungicidal sensitivity of the strawberry powdery mildew fungus has not been investigated. This method will be useful for screening of fungicide sensitivity and for developing disease control strategies.

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/ 和 文 摘 要

岡山健夫・中野智彦・松谷幸子・杉村輝彦：イチゴうどんこ病に対する薬剤の効果を簡易かつ確実に評価できる試験法

イチゴうどんこ病に対する薬剤の効果を簡易に評価できる試

験法を考案した。うどんこ病菌の接種にはランナー先端小葉が適し，接種7日後に葉裏の菌そうを肉眼観察して発病程度を判定できた。展開直後の若葉が最も高率に発病し，成葉化するにつれて発病小葉率が低下した。接種7日後に菌そうが肉眼観察でき，4週間生存した。接種胞子濃度は5.0×10^4/ml以上で高率に発病し，発病程度も安定した。接種後の温度条件は20°Cで高率に発病し，次いで15°C，25°Cの順に発病程度が低下した。25°Cでは発病程度が抑えられ，30°Cでは発病しなかった。有効薬剤をあらかじめ処理したランナー先端小葉は発病が抑制され，薬剤の防除効果が適切に評価でき，薬剤および濃度間の効果の差異を明確にすることができた。切断先端小葉を用いた試験管内試験における薬剤効果の発現は，圃場試験よりも明瞭であり，簡易かつ迅速な薬剤効果の評価に適している。