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AWARD

Integrated disease management of strawberry anthracnose and development of a new biopesticide

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Introduction

In Japan, strawberry anthracnose caused by *Glomerella cingulata* (Stoneman) Spaulding et Schrenk and *Colletotrichum acutatum* Simmonds is regarded as a serious disease of strawberry (*Fragaria* × *ananassa*). Since the 1980s, with the introduction of new cultivars (Nyohou and Toyonoka), the disease has become more serious in many areas in Japan. Infection by *G. cingulata* has caused severe damage to the strawberry industry in the main production areas of Tochigi Prefecture, the largest producer of strawberry fruits for 44 years, causing many farmers to stop growing strawberry. To address this problem, I have studied the (1) ecology and epidemiology of two anthracnose pathogens; (2) practical application of biological control of disease using antagonistic fungi; and (3) development of a simple diagnostic method using an ethanol immersion treatment (SDEI).

The results of these studies, which have already been adopted for the development of a standard method for eco-friendly control of strawberry anthracnose in several countries including Japan, will be presented here.

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Epidemiology and integrated disease management of strawberry anthracnose

Symptoms and epidemiology

Manifestations of strawberry anthracnose caused by *G. cingulata* and *C. acutatum* include spotting and girdling of stolons and petioles, crown rot, fruit rot, and black leaf spot (Ishikawa et al. 1989a, 1992; Matsuo and Ota 1992).

Colletotrichum acutatum, a new causal fungus of strawberry anthracnose, was first found in Tochigi and Nagasaki prefectures in 1991 (Chikuo et al. 1992; Ishikawa et al. 1992; Matsuo and Ota 1992). This fungus has not been associated with crown rot although it was observed in Hokkaido, Japan in 2006 (Misawa et al. 2010). The most damaging of these diseases is anthracnose crown rot caused by *G. cingulata*.

Glomerella cingulata can overwinter in infected strawberry stocks and as perithecia on strawberry residues, which can then serve as inoculum to initiate the first infection in a strawberry nursery (Horn and Carver 1968; Ishikawa et al. 1989b). Conidia of *C. gloeosporioides* (*G. cingulata*) are effectively dispersed by water and wind. Although the conidia are barely blown off by wind in the absence of water, when in contact with water, they become suspended in the water droplets, which are then splashed by wind, dispersing the conidia (Ishikawa et al. 1993).

Cultural control

Plastic-film rain shelters and capillary watering systems in nursery beds help prevent dispersal of conidia and control the incidence of strawberry anthracnose (Ishikawa 2005; Ishikawa et al. 1989c; Okayama 1993). In addition, debris of diseased plants anaerobically ferments when sealed in

plastic film bags, thus effectively inactivating *G. cingulata* under the anaerobic conditions (Hagiwara 1983; Ishikawa 2005; Ishikawa et al. 1990).

Simple diagnostic method by ethanol immersion (SDEI)

The causal fungi of strawberry diseases, *G. cingulata*, *C. acutatum*, *Dendrophoma obscurans* and *Fusarium oxysporum* f. sp. *fragariae*, can remain latent for a long time in infected strawberry plants without causing visible symptoms, but later serve as primary inoculum (Eshenaur and Milholland 1989; Horn and Carver 1968; Inada et al. 1998; Ishikawa et al. 1989b; Makino et al. 1982).

Asymptomatic latent infections in strawberry leaves can be detected by inducing the various fungi to sporulate using SDEI (Ishikawa 2003, 2004a). In the SDEI treatment, sampled leaflets are thoroughly in running tap water, then immersed in 70 % ethanol for 30 s and rinsed twice in sterile distilled water (SDW) under aseptic conditions. Each leaflet is then placed on a filter paper moistened with SDW in a Petri dish with the treated surface up and incubated at 28 °C in the dark (Ishikawa 2003). The color of SDEI-treated leaves changes to dark brown or nearly black, and salmon-pink conidial masses are subsequently produced in the acervuli after 5 to 10 days. With SDEI, conidial mass formation is greater on leaves from lower on the plant. The time required for conidial production or the rate of conidial formation, however, was the same regardless of the causal isolate, strawberry cultivar, or leaf position, nor did varietal differences in resistance to strawberry anthracnose influence the rate of formation.

Similarly, signs on strawberry leaves with asymptomatic latent infection by *C. acutatum* can also be seen after SDEI; again, after 5 days at 28 °C, salmon pink conidial masses produced in acervuli are visible (Ishikawa 2004a). For *D. obscurans* using the SDEI treatment, pycnidia with amber conidial masses form after 5 days at 28 °C (Ishikawa 2004a). The pycnidia form mainly on the leaf ribs, and macroscopically visible masses, similar to those of *G. cingulata* and *C. acutatum*, exude from the ostiole. When water is dribbled onto the lesions, the pycnidia exude white, filamentous conidial masses, differentiating it from *G. cingulata*. In the case of petioles latently infected by *F. oxysporum* f. sp. *fragariae*, white aerial hyphae, which are easily visible by eye or with a loupe, grow out from the vascular tissues on the cut surface after the SDEI treatment and 3 days incubation at 28 °C (Ishikawa 2004a).

These results illustrate how useful the SDEI can be for selecting healthy mother plants and its potential for forecasting the incidence of strawberry anthracnose and fusarium wilt (Ishikawa 2004b).

Development of a new biopesticide

The fungus *Talaromyces flavus* has been shown to suppress verticillium wilt of eggplant and to increase yield of eggplant in commercial fields (Fravel et al. 1995; Marois et al. 1982). However, no biopesticide of *T. flavus* has been developed to control strawberry anthracnose.

Strains of *T. flavus* were isolated from strawberry crowns and screened for their ability to inhibit anthracnose in the nursery. All 13 tested strains were found to strongly suppress the disease when the plants were pretreated with the suspension of the test strains; SAY-Y-94-01 was most inhibitory activity equivalent to Propineb. This strain is an effective biological agent to control the following diseases and their pathogens: strawberry anthracnose (*G. cingulata*); powdery mildew (*Sphaerotheca aphanis* var. *aphanis*); rice diseases such as "Bakanae" disease (*Gibberella fujikuroi*), blast (*Magnaporthe oryzae*), bacterial seedling blight (*Burkholderia plantarii*), bacterial grain rot (*B. glumae*), bacterial brown stripe (*Acidovorax avenae* subsp. *avenae*) that are caused by seed-borne pathogens; and tomato leaf mold (*Fulvia fulva*) (Ishikawa 2005). A conidial formulation of SAY-Y-94-01 was developed by the Idemitsu Kosan Co., Ltd. and is sold as: Biotrust WP (Registered No 20659, in 2002), Toughpearl (Registered No 21919, in 2007), Toughblock (Registered No 21919, in 2007), and Toughblock SP (Registered No 23054, in 2012). These biopesticides account for a 35 % share of the biopesticide market in Japan.

Japanese paddy-rice seedlings grown in nursery boxes offer a greater advantage for the biocontrol of soil-borne and seed-borne pathogens compared with those in soil culture. Toughblock and Toughblock SP were further developed to control seed-borne pathogens of rice diseases such as "Bakanae" disease, blast, bacterial seedling blight, bacterial grain rot, other bacterial and fungal diseases of nursery rice plants. Both biopesticides have suppressive effects nearly equivalent to ipconazol-copper flowable for "Bakanae" disease and to oxolinic acid for bacterial seed-borne pathogens (Nojo et al. 2012).

In dual culture on water agar, hyphae of strain SAY-Y-94-01 grow toward and coil around hyphae of the pathogen (Ishikawa 2005). Tips of hyphal branches often invade the fungal host by directly penetrating the cell wall. Infection of fungal host cells by *T. flavus* resulted in granulation of cytoplasm and collapse of the cell. *Trichoderma flavus* is a destructive hyperparasite of *G.* cingulata, *C. acutatum*, *S. aphanis* var. *aphanis*, *Botrytis cinerea*, *F. oxysporum* f. sp. *fragariae*, *Rhizoctonia solani*, *Phytophthora cactorum*, *Sclerotium rolfsii*, and many other fungi (Ishikawa 2005; McLaren et al. 1986).

A lignin-like substance forms in the leaf and root tissues of strawberry 3 days after they are sprayed with conidia of

T. flavus SAY-Y-94-01. This substance is rare in non-treated strawberry tissues (Ishikawa 2005). Because lignification of plant cell walls is a common mechanical defense reaction of plants in response to infection (Asada and Matsumoto 1967, 1969), lignin may be the most effective barrier to hyphal growth after infection.

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