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Interaction between Allelochemicals and *Fusarium* Root Rot in Asparagus Seedlings Cultured *In Vitro*

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Abstract

The interaction between *Fusarium* root rot and 4 allelochemicals in asparagus seedlings was estimated *in vitro* to clarify the relationship between biotic and abiotic factors in asparagus decline. In *in vitro* culture of *Fusarium oxysporum* f. sp. *asparagi* (Foa) with or without addition of 4 allelochemicals (caffeic acid, ferulic acid, quercetin, malic acid; 0.01%, 0.1%, w/v) using Czapec-Dox media, Foa propagation was suppressed in all the treatments. The degree of suppression became higher in 0.1% than 0.01% among all the allelochemicals. As for the axenic culture of asparagus (*Asparagus officinalis* L., "Welcome") seedlings with the 4 allelochemicals, dry weight of both shoots and roots became lower compared to control in 0.1% and 0.01% of caffeic acid, 0.1% ferulic acid, 0.01% quercetin, only dry weight of shoots decreased in 0.1% malic acid. Two weeks after Foa inoculation with Foa-cultured PDA cubes *in vitro*, incidence of *Fusarium* root rot reached 100% in most of the plots. The severity of root rot increased in 0.01% and 0.1% caffeic acid, 0.1% ferulic acid, 0.1% malic acid compared to control. From these results, the 4 allelochemicals used in this study are supposed to suppress asparagus growth, and such growth reduction might enhance the disease severity of *Fusarium* root rot as an indirect effect. In addition, such effect might differ with the allelochemicals and concentrations in asparagus.

Keywords

Fusarium oxysporum f. sp. *asparagi*, Spore Propagation, Caffeic Acid, Ferulic Acid, Disease Severity

1. Introduction

Asparagus decline is a serious and increasing threat in asparagus-producing regions over the world [1] [2] [3] [4]. It is supposed to be caused by the contribution of both biotic (disease) factors [2] [3] and abiotic (allelopathy etc.) factors [5] [6] [7]. As biotic factors, the most common phenomenon is *Fusarium* crown and root rot caused mainly by *Fusarium oxysporum* f. sp. *asparagi*, *Fusarium proliferatum* and *Fusarium redolence*, etc. [2] [3] [4] [8]. Nahiyan *et al.* [9] demonstrated that *Fusarium oxysporum* f. sp. *asparagi* and *Fusarium proliferatum* are dominant in asparagus decline fields in Japan by PCR-SSCP (single-stranded conformational polymorphism) analysis. However, it is difficult to control these diseases successfully with cultural and chemical methods due to the perennial nature of the crop, the pathogens are soil-borne, and highly resistant cultivars have not been developed [4] [10].

Abiotic factors are mainly related to the release of allelochemicals; nutrient imbalance, deterioration of soil physiochemical conditions, cultural factors, and excessive harvesting pressure were reported [1] [5] [6] [7] [11] [12]. As for the allelochemicals, caffeic acid, ferulic acid, quercetin, malic acid are reported as asparagus root origin [5] [6] [13] [14]. On the other hand, there have been many reports on *Fusarium* diseases or allelochemicals in concern with asparagus decline. However, it has been still unclear the interaction between *Fusarium* diseases and allelochemicals. Wu *et al.* [15] [16] [17] reported that allelochemicals of watermelon, such as coumarin, caffeic acid, ferulic acid, salicylic acid, cinnamic acid, have inhibitory effects on growth of *Fusarium oxysporum* f. sp. niveum *in vitro*. These reports are estimated only the direct effect of allelochemicals on *Fusarium* growth *in vitro*. In addition, it is still unclear the direct effect of asparagus allelochemicals on propagation of *Fusarium oxysporum* f. sp. *asparagi*, and the interaction between disease severity and allelochemicals in asparagus plants.

In this study, the interaction between *Fusarium* root rot and 4 allelochemicals in asparagus seedlings was estimated *in vitro* to clarify the relationship between biotic and abiotic factors in asparagus decline.

2. Materials and Methods

2.1. Foa Culture with Allelochemicals *In Vitro*

The conidia of *Fusarium oxysporum* f. sp. *asparagi* (Foa, MAFF305556) grown on PDA media was subcultured for 2 weeks on Czapec-Dox media containing $NaNO_3$ 3 g, K_2HPO_4 1 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, sucrose 30 g, agar 8 g·L^{-1} (pH 5.8). The conidia were further subcultured (10^6 conidiam L^{-1}) in liquid Czapec-Dox media with or without addition of filter-sterilized allelochemicals (caffeic acid, ferulic acid, quercetin, malic acid; 0.01%, 0.1%, w/v) at 25˚C in the dark for one week by shaking culture (100 rpm). Then, the density of conidia was investigated using hemocytometer and calculated the propagation index of allelochemical-added plots to allelochemi-

cal-non-added plots. The average was calculated from 10 replications.

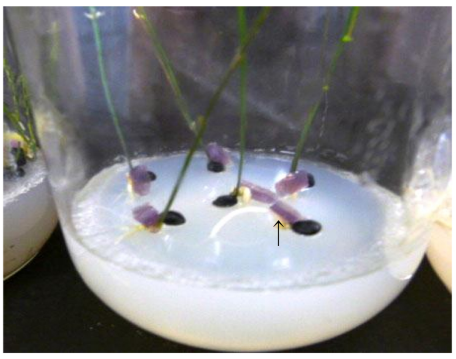
2.2. Plant Culture with Foa and Allelochemicals *In Vitro*

Seeds of asparagus (*Asparagus officinalis* L., "Welcome") were axenically sown to Knop's medium [18] with filter-sterilized allelochemicals (caffeic acid, ferulic acid, quercetin, malic acid; 0.01%, 0.1%, w/v, using ultra pure water with few ethanol) and cultured in the growth chamber (25˚C, 16 hr daylength). Four weeks after sowing, Foa (MAFF305556)-cultured PDA cubes (5 × 5 × 5 mm) were placed on the proximal part of the roots (**Figure 1**). Ten plants with two replications were used. Two weeks after Foa inoculation, the symptoms were categorized into 5 degrees: percentage of root lesion length to total root length in a root system: 1, less than 20%; 2, 20% - 40%; 3, 40% - 60%; 4, 60% - 80%; 5, 80% - 100%. The index (IDL; index of diseased length to total root length) was calculated by the following formula:

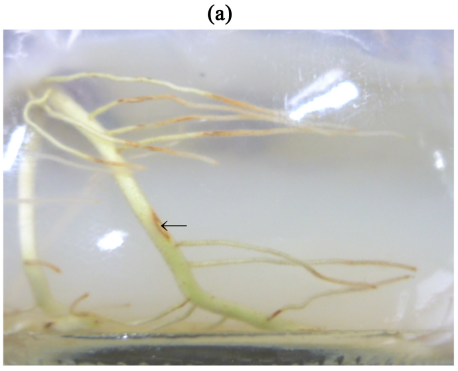
$$\text{IDL index} = \frac{\sum(\text{number of plants} \times \text{number of degree in symptom})}{\text{Total number of plants} \times 5(\text{maximum degree in symptom})} \times 100$$

2.3. Statistical Analysis

The data were compared by Tukey's test at $P < 0.05$. All analyses were performed



(a)



(b)

Figure 1. Inoculation of Foa (a) and root lesions in asparagus seedlings *in vitro* (b) Foa; *Fusarium oxysporum* f. sp. *asparagi*. (a) Arrow is agar cube of Foa; (b) Arrow is root lesion caused by Foa inoculation.

using XLSTAT pro statistical analysis software (Addinsoft, New York).

3. Results

In the *Fusarium oxysporum* f. sp. *asparagi* (Foa) culture with allelochemicals *in vitro*, Foa propagation was suppressed in all the treatments; the degree of suppression differed with the combinations between allelochemicals and concentrations (Figure 2). In this case, the suppression effect of Foa propagation in 0.1% plots became higher than 0.01% plots among all the allelochemicals.

Four weeks after sowing *in vitro*, dry weight of asparagus seedlings differed among the plots with the combination of allelochemicals and concentrations (Figure 3). Dry weight of both shoots and roots became lower compared to control in the plots added with 0.1% and 0.01% caffeic acid, 0.1% ferulic acid, 0.01% quercetin, only dry weight of shoots in 0.1% malic acid. In this case, suppression of shoot and root length occurred in such plots (data not shown). No growth suppression in shoots and roots occurred in 0.01% ferulic acid, 0.1% quercetin and 0.1% malic acid.

Two weeks after Foa inoculation, incidence of *Fusarium* root rot showed 100% in most of the plots except ferulic acid with 0.01% and 0.1%, 0.1% quercetin and 0.1% malic acid (Figure 4). However, the ratio of diseased roots increased in 0.01% and 0.1% caffeic acid, 0.1% ferulic acid, 0.1% malic acidcompared to control. Index of diseased length became higher compared to control in 0.01% and 0.1% caffeic acid, 0.1% ferulic acid, 0.01% qercetin and 0.1% malic acid (Figure 5). In this case, IDL reached more than 60 in 0.1% of caffeic acid and ferulic acid.

4. Discussion

In this study, 4 allelochemicals with different concentration levels showed

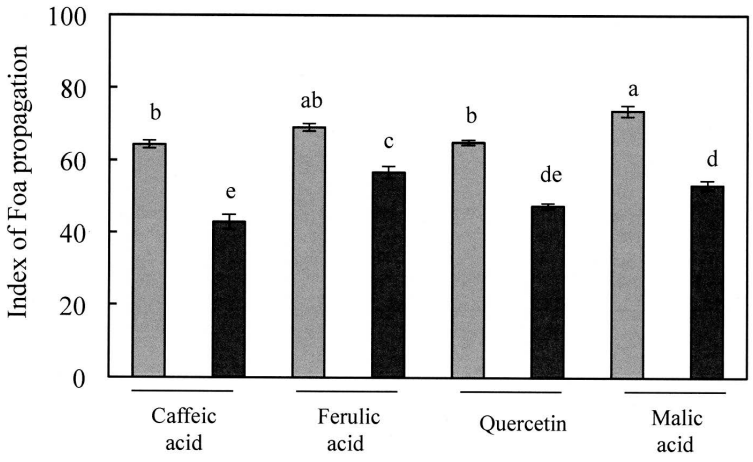


Figure 2. Influence of allechemicals on propagation of Foa (*Fusarium oxysporum* f. sp. *asparagi*; MAFF305556). ▒, 0.01%, ■, 0.1%. Bars represent standard errors (n = 10). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

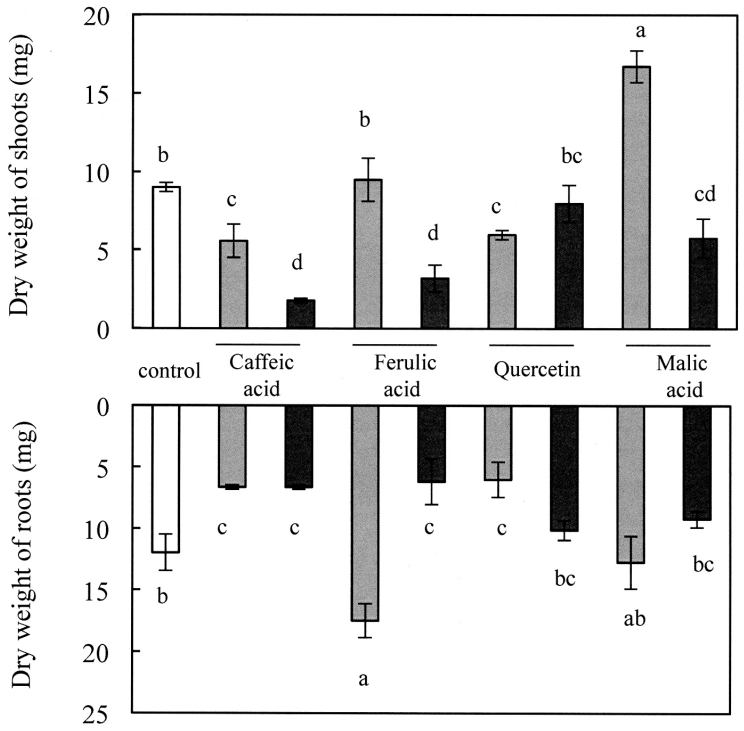


Figure 3. Dry weight of asparagus seedlings 4 weeks after sowing in allelochemical-added Knop's media. □, control; ▒, 0.01%; ■, 0.1%. Bars represent standard errors (n = 10). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

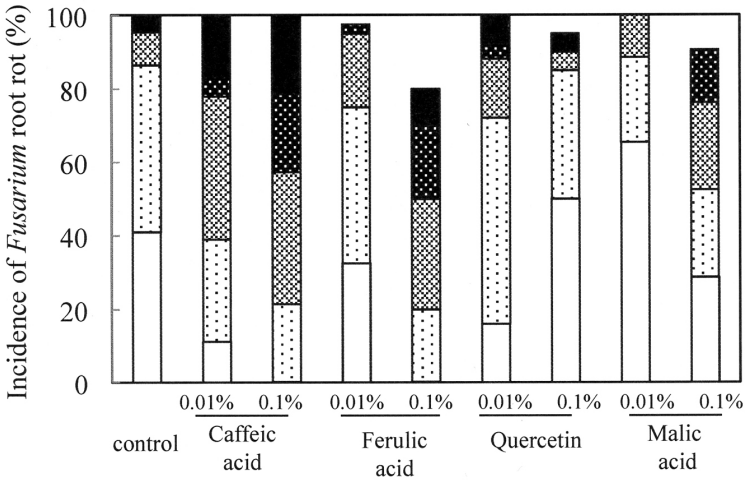


Figure 4. Incidence of *Fusarium* root rot in asparagus plants 2 weeks after *Fusarium oxysporum* f. sp. *asparagi* (MAFF305556) inoculation. Ratio of diseased storage roots in a root system; □, −20%; ⁙, 20% - 40%; ▒, 40% - 60%; ▓, 60% - 80%; ■, 80% - 100%.

suppression effect in Foa propagation *in vitro*. Wu *et al.* [15] [16] [17] [18] reported that allelochemicals in watermelon, such as caffeic acid, ferulic acid, salicylic acid, cinnamic acid, have inhibitory effects on growth of *Fusarium oxysporum* f. sp. *niveum in vitro*. In their reports, the suppression effect increased

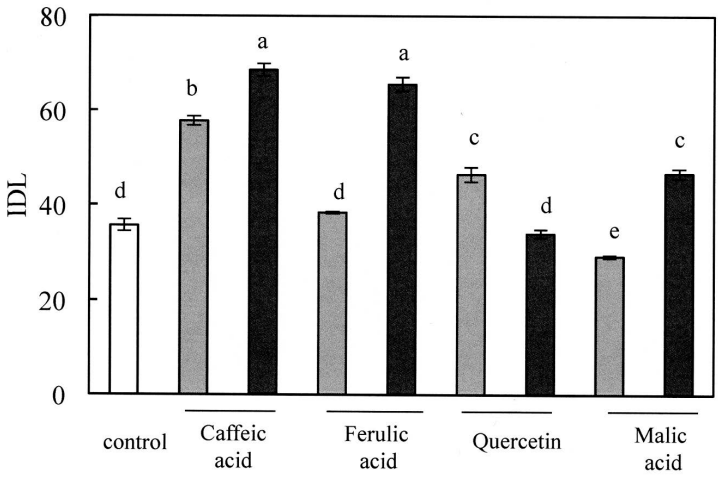


Figure 5. Index of diseased length to total root length (IDL) in asparagus plants cultured by allelochemical-added media. □, control; ▒, 0.01%; ■, 0.1%. Bars represent standard errors (n = 10). Columns denoted by different letters indicate significant difference according to Tukey's test ($P < 0.05$).

along with the concentration of allelochemicals added. In this experiment, same phenomena occurred in the 4 asparagus allelochemicals, so that Foa growth itself might not be directly enhanced by such 4 allelochemicals.

In the present study, some allelochemical-added plots induced growth reduction in shoots and roots before Foa inoculation, and the severity of *Fusarium* root rot increased in such growth-restricted plots after Foa inoculation. In this case, growth reduction and increase in disease severity greatly appeared especially in caffeic acid-added plots. Miller *et al.* [6] reported that some allelochemicals suppressed seed germination of asparagus, Inotani *et al.* [19] mentioned cinamic acid, oxalic acid and ferulic acid induced lower germination rate in lettuce etc. Chaves *et al.* [20] reported suppression of germination and seedling growth in *Cistus ladanifer* differed with the kinds of eleven allelochemicals added. Gisele *et al.* [21] reported root growth reduction by the addition of caffeic acid in soybean (*Glycine max*). From these findings, the four allelochemicals used in this study are supposed to suppress asparagus growth by some physiological obstacles under allelochemical-accumulated conditions, and such growth reduction might enhance the disease severity of *Fusarium* root rot as an indirect effect. In addition, such effect might differ with the allelochemicals and concentrations in asparagus. From these findings, this study firstly reported asparagus allelochemicals on propagation of *Fusarium oxysporum* f. sp. *asparagi,* and the interaction between disease severity and allelochemicals in asparagus plants. On the other hand, Wu *et al.* [15] [16] [17] reported that several allelochemicals of watermelon inhibited the growth of *Fusarium oxysporum* f. sp. *niveum*, however, such allelochemicals stimulated the mycotoxin (mainly fusaric acid) production of *Fusarium in vitro*. From these reports, it is supposed that asparagus allelochemicals might also enhance mycotoxin production of Foa, resulting in the

increase of disease severity in asparagus plants. Further study would be needed on this point.

In this experiment, the tested allelochemicals have been already reported as asparagus root origin or root extract-included chemicals [6] [13] [14] [19]. In addition, the chemical concentrations in this study were almost the same levels as the reports of Wu *et al.* [16] [17]. However, it has been still difficult to estimate the real concentrations of such allelochemicals in the practical asparagus decline fields. On the other hand, Wu *et al.* [22] described that some amino acids such as alanine containing in cotton root exudates promoted the growth of *Verticillium dahliae*. Hao *et al.* [23] mentioned that alanin containing in root exudates of watermelon promoted spore germination and sporulation of *Fusarium oxysporum* f. sp. *niveum in vitro*. In this study, the author established the model method to estimate the chemical effect on *Fusarium* propagation and bioassay method using asparagus seedlings *in vitro*. Further investigation using the method established in this study would be needed to clarify the relationship between other secondary metabolites including amino acids and biotic factors on asparagus decline.

5. Conclusion

In this study, 4 allelochemicals (caffeic acid, ferulic acid, quercetin, malic acid; 0.01%, 0.1%, w/v) suppressed propagation of *Fusarium oxysporum* f. sp. *asparagi* (Foa) *in vitro*. As for the axenic culture of asparagus seedlings with the 4 allelochemicals, dry weight of both shoots and roots became lower compared to control, and disease severity of *Fusarium* root rot increased in such growth-suppressed plots. The 4 allelochemicals used in this study are supposed to suppress asparagus growth, and such growth reduction might enhance the disease severity of *Fusarium* root rot as an indirect effect. In addition, such effect might differ with the allelochemicals and concentrations in asparagus. This study established the model method to estimate the allelochemical effect on *Fusarium* propagation and bioassay method using asparagus seedlings *in vitro*. Further investigations using these methods would clarify the relationship between chemical and biotic factors on asparagus decline.

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