Role of Conifer Litter in Ecology of Fusarium: Stimulation of Germination in Soil

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ABSTRACT

Water extracts of ponderosa pine, sugar pine, white fir, and coast redwood litter stimulated the germination of macroconidia and chlamydospores of *Fusarium oxysporum*. Germination of chlamydospores of *F. oxysporum*, *F. oxysporum* f. sp. *vasinfectum*, *F. solani*, and *F. solani* f. sp. *phaseoli* was also enhanced in conifer soils, and reached 95 to 98%. When ponderosa litter extract was compared with 1% glucose and 2.5% asparagine solutions on chlamydospores of *F. oxysporum* in soil, greater germination was obtained with the litter extract. But in conifer soils, the germination generally resulted in the production of abnormal germ tubes that lysed rapidly before new chlamydospores were formed. It is postulated that germ tube lysis of such a large proportion of the population may account, in part, for the general absence of *Fusarium* spp. in conifer forest soils in California. Phytopathology 59:1396-1399.

Smith (4) reported that isolates of *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. which are commonly present in the rhizosphere of sugar pine (*Pinus lambertiana* Dougl.) seedlings in California forest nurseries were absent on native seedlings. He also showed that the *Fusarium* population declined rapidly when nursery seedlings were transplanted to a native pine forest in the Sierra Nevada. The present work was undertaken to determine the cause of this decline.

METHODS AND RESULTS.—Soil surveys for *Fusarium* spp. were made in forests in Stanislaus, Placer, and Lassen Counties, using Nash & Snyder's isolation medium (2). No *Fusarium* was isolated from soil obtained from pine stands of these forests or from pine stands on the outskirts of Berkeley. However, surveys made in Stanislaus and Placer Counties in brush and grassland areas contiguous to the sampled pine forest soils yielded colonies of *F. oxysporum* and *F. roseum* Link emend. Snyd. & Hans. Since climate and soil were probably not the controlling factors, we examined the heavy layer of needles under conifer trees.

An extract was obtained by soaking 100 g of needles (air-dry wt) in 1,000 ml of H_2O for 10 days at 10 C. The clear extract was centrifuged to remove debris, and frozen until used. Water extracts were also prepared from the mineral soil, following the method of Alexander et al. (1). All experiments were done in the laboratory at room temperature (21-25 C), either by placing the extracts and macroconidia (from potato-dextrose agar cultures) together in well-slides (in vitro tests), or by adding the extracts to nonsterile soils in which chlamydospores had previously formed (in vivo tests). One hundred propagules were counted at random in each experiment. All experiments were replicated three times.

Macroconidia of a clone of *F. oxysporum* isolated from moribund pine seedlings were exposed in vitro to sugar pine needle extracts. Extracts obtained from the top layer of undecomposed needles (litter), the layer of partially decomposed needles (duff), and the bottom layer of completely decomposed needles (humus) stimulated germination of 95-98% of the spores as compared to 50% in distilled water. The same results were obtained with macroconidia of a second clone of *F. oxysporum* and a clone of *F. solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. isolated from nursery soil. The experiments were repeated with chlamydospores produced from macroconidia by the method of Alexander et al. (1) with the same results. Extracts of the litter of ponderosa pine (*P. ponderosa* Laws.), white fir (*Abies concolor* [Gord. & Glend.] Hoopes), and coast redwood (*Sequoia sempervirens* [Lamb.] Endl.) gave germination percentages of 96-98 as compared to 30% in distilled water.

The effect of ponderosa litter extract was then determined on chlamydospores in nonsterile soil. For this and all following experiments, conidia of *F. oxysporum*, *F. solani*, *F. oxysporum* f. sp. *vasinfectum* isolated from cotton in Tulare Co., and *F. solani* f. sp. *phaseoli* isolated from beans in Monterey Co. were added to pine soils from two locations in the Sierra Nevada, and to a Tulare Co. cotton-field soil and a Monterey Co. bean-field soil. The conidia were allowed to convert to chlamydospores. The soils were then moistened with the extracts. The germination of the chlamydospores and their subsequent behavior were observed under the microscope by means of soil smears stained with cotton blue in water.

Very high germination was obtained with the nonpathogenic clone of *F. oxysporum* in all soils (Table 1),

Fig. 1. Germinated chlamydospores of *Fusarium oxysporum* in pine forest soil, Stanislaus Co., after ponderosa pine litter extract was added (except E, which germinated with the addition of a 2.5% asparagine solution). Stained with cotton blue in water. **A, B)** Lysis of germ tubes soon after germination. **C)** Lysis of older germ tubes; in such cases all cells generally lyse simultaneously. **D)** Abnormal morphology of germ tubes frequently noted before lysis; the germ tube is misshapen and constricted toward its tip. **E)** Robust, branching germ tube after germination with the addition of a 2.5% solution of asparagine. New chlamydospores will ultimately be produced. Similar results were obtained with a 1% glucose solution. (A, B, C, D approximately ×720, E approximately ×330.)

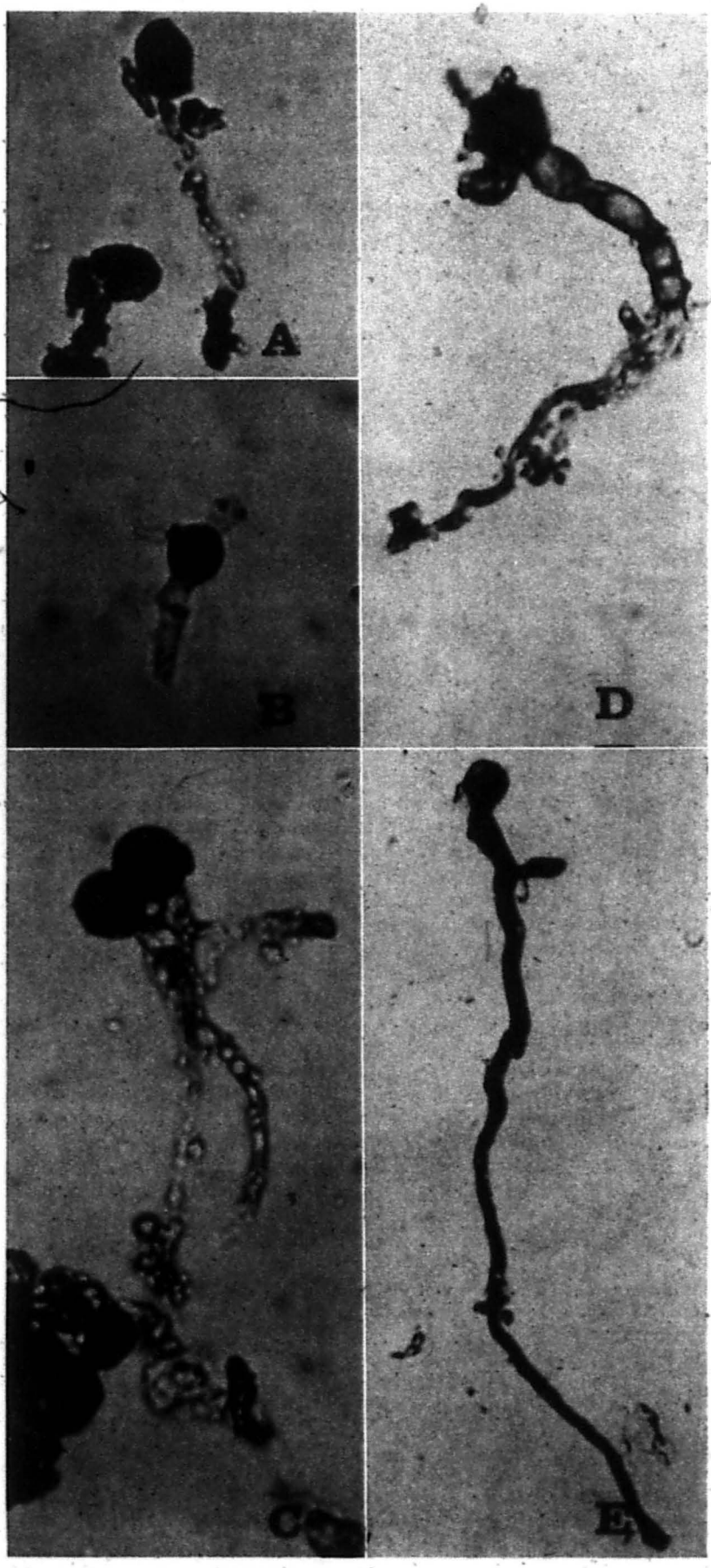
A
B
C
D
E

TABLE 1. Effect of ponderosa pine litter extract on chlamydospore germination of four fusaria in forest and agriculture soils from four localities in California

Soil	County	Chlamydospore germination			
		F. oxysporum[a]	*Fusarium oxysporum* f. sp. *vasinfectum*[b]	*F. solani*[a]	*F. solani* f. sp. *phaseoli*[c]
		%[d]	%	%	%
Bean	Monterey	87	55	19	0
Cotton	Tulare	90	68	5	17
Pine soil A.	Stanislaus	97	98	86	91
Pine soil B.	Placer	95	97	93	93

[a] Isolated from forest nursery soil, Placer County.
[b] Isolated from cotton fields, Tulare County.
[c] Isolated from bean fields, Monterey County.
[d] Each figure based on a random count of 100 chlamydospores.

and with all the *Fusarium* spp. tested in the forest soils. Although high germination was obtained with the pathogenic *F. oxysporum* in the agricultural soils, the germination percentages of the two clones of *F. solani* dropped considerably in these soils.

The very high germination of *F. oxysporum* chlamydospores in forest soils prompted experiments in which the effect of ponderosa pine litter extract was compared to that of a 1% solution of glucose and a 2.5% solution of asparagine. These nutrients are known to stimulate *F. oxysporum* germination in soil. The results again suggest that almost all of the chlamydospores germinated under the influence of the litter extract (Table 2). The percentage was clearly greater than that obtained with the two nutrients, particularly when the agricultural soils are compared.

The high germination rate (96%) of *F. oxysporum* chlamydospores in forest soils continued even when the concentration of the litter extract was increased ten times. A tenfold dilution of the extract (0.1% of the original extract) resulted in a germination of 21%; a hundredfold dilution gave 10% germination. Distilled water controls gave 3% germination.

In forest soils, the germ tubes of *F. oxysporum* lysed before new chlamydospores could be formed. This destruction could occur very soon after germination (Fig. 1-A, B). In older germ tubes all cells can be lysed simultaneously (Fig. 1-C). The majority of the germ tubes appeared to be abnormal before lysis, in that they markedly decreased in diameter toward the tip (Fig. 1-D). This behavior was in contrast to that obtained when glucose or asparagine solutions were added to the soil. Here the growth was normal and vigorous, and lysis did not occur until new chlamydospores had formed, at least.

DISCUSSION.—In attempting to understand the reason for the rapid decline of *F. oxysporum* populations introduced into conifer soils, and the almost universal absence of *Fusarium* in such soils (3, 5), we have obtained, by the use of pine needle leachates, the highest percentage of *Fusarium* chlamydospore germination that we have ever recorded. This activity involved almost the whole population under study. This germination was accompanied by the formation of abnormal germ tubes which were destroyed before new chlamydospores were formed. This condition did not exist when germination-stimulating nutrients, such as glucose or asparagine, were added to the same soils. It remains to be seen, however, whether chlamydospore germination and the destruction of germ tubes before the formation of new chlamydospores occur in the forest to the same extent as it was observed in the laboratory, and are the reasons for the absence of fusaria in conifer soils. We hope that our continuing studies on populations in the field will clarify this.

The concentration of the extract we used corresponds to 20 square inches (129 cm^2) of litter, through which 60 inches (152 cm) of rain have percolated. This amount does not seem to be excessive as far as conditions in the Sierra Nevada are concerned, since the needle layer is often 15 cm deep and is wet during winter under snow cover.

Forest soils in general supported higher chlamydospore germination than did the agricultural soils tested. The biotic and abiotic factors which may be responsible for this difference, as well as the nature of the active

TABLE 2. Effect of amendments on germination of *Fusarium oxysporum* chlamydospores in forest and agricultural soils from four localities in California

Soil	County	Chlamydospore germination/amendment			
		Ponderosa litter extract	1% glucose	2.5% asparagine	Distilled water
		%[a]	%	%	%
Bean	Monterey	85	19	7	3
Cotton	Tulare	94	21	28	2
Pine soil A.	Stanislaus	98	63	72	5
Pine soil B.	Placer.	95	62	80	2

[a] Each figure based on a random count of 100 chlamydospores.

principle(s) in litter extracts, are now included as part of our investigation.

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