# The Effects of Growth Regulating Compounds on Healthy and Blister Rust-Infected Tissue Cultures of Pinus monticola

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## ABSTRACT

With increasing concentration, indoleacetic acid (IAA) (0.1-10.0 mg/liter), napthaleneacetic acid (NAA) (0.01-1.0 mg/liter), or 2,4-dichlorophenoxyacetic acid (2,4-D) (0.01-10.0 mg/liter) progressively enhanced growth of both *Pinus monticola* and *Cronartium ribicola* in tissue culture. At concentrations above 3 mg/liter, 2,4-D inhibited fungus growth. Kinetin, in combination with these auxins (1/1 to 1/10 ratio), did not affect growth but did increase vigor and longevity of both organisms. The high auxin levels (above), particularly in combination with kinetin, induced formation of aecialike bodies by the pathogen. Gibberellic acid (0.01-1.0 mg/liter) had no observable effect on host tissues, but inhibited both the growth of the fungus and its formation of aecialike bodies. High concentration of kinetin (1.0-5.0 mg/liter), in combination with high sugar levels (2-5%) in tissue culture media, stimulated formation of a red pigment in the surface layers of host tissues, and subsequently caused reduction in the growth of both host and fungus. Phytopathology 61:507-509.

*Additional key words:* gibberellin, host-parasite interactions.

The effects of plant growth regulators on fungi have been intensively investigated. Many reports have noted a positive relationship between changes in fungus growth and morphology and exogenously applied growth regulators (2). Plant growth regulators have been implicated in the host-parasite relations of many rusts (11, 12, 13, 14, 15, 16, 17). They have been specifically related to the development of the expression of resistance to, and symptoms of, western white pine blister rust (1). In addition, we have previously related high concentrations of growth regulators to enhanced mycelial growth and to aborted attempts to produce aeciospores in host tissue cultures (4, 9).

Our recent successes in culturing the mononucleate phase of *Cronartium ribicola* J. C. Fisch. ex Rabenh. on tissue cultures and/or tissue culture diffusates from *Pinus monticola* Dougl. provide several methods of obtaining mycelia from this rust under defined conditions (3, 4, 5, 6, 7, 8, 9, 10). Propagation of *C. ribicola* on host tissues growing in vitro (3, 4) was chosen as an experimental tool. This enabled us to examine effects of plant growth regulators on (i) the growth and differentiation of both healthy and blister rust-infected western white pine tissue cultures; and (ii) the direct and/or indirect influence of these regulators on the growth and morphology of the blister rust fungus.

MATERIALS AND METHODS.—All healthy pine tissues used in this study were taken from 6-0, open-pollinated nursery stock. Infected tissues were derived from 2- to 3-year-old rust infections randomly collected from heavily infected young stands of western white pine in the St. Joe National Forest, Idaho.

Primary explants were prepared and set by techniques and media previously described (3, 4). Growth regulators, singly or in various combinations and concentrations, were filter-sterilized and incorporated into the basal medium (3) to evaluate their effects on the host, the parasite, and on the host-parasite combination.

Test media were adjusted to a pH of 5.5 and added, in 15-ml aliquots, to 100 × 25 mm test tubes sealed with a single layer of plastic food wrap. All cultures were incubated under 24-hr cyclic conditions; i.e., 16 hr at 21 C with 400 ft-c of fluorescent light, and 8 hr in darkness at 5 C for 90 days. Conclusions regarding the effects of these compounds were based on actual measurements and/or on macroscopic observations of at least 25 explants from any given experiment, and microscopic observations on randomly selected explants from each experiment.

RESULTS.—*Single growth regulator effects.*—The single auxins, indoleacetic acid (IAA) (0.1-10.0 mg/liter), napthaleneacetic acid (NAA) (0.01-1.0 mg/liter), and 2,4-dichlorophenoxyacetic acid (2,4-D) (0.01-10.0 mg/liter), when added to the basal medium, induced proliferation and growth of both healthy and blister rust-infected cortex explants of western white pine. Auxin effects on both types of tissue were similar. Indoleacetic acid, (1.0 mg/liter), NAA (1.0 mg/liter), and 2,4-D (0.1 mg/liter) produced vigorous growth of host tissues (Table 1). However, by increasing the concentrations of these auxins to 10 mg, 10 mg, and 1.0 mg/liter, respectively, we progressively enhanced fungus growth on infected tissue (Table 1) and induced formation of aecialike bodies without spores (4). Three mg/liter 2,4-D inhibited fungus growth. Two other auxins, indoleproprionic acid and indolebutyric acid, had no effect on these tissues at any concentration tested (0.01-1.0 mg/liter).

Singly, or as an addition to an auxin-containing medium, Gibberellic acid (GA) (0.1-1.0 mg/liter) had no observable effect on healthy host tissues. With blister rust-infected tissues growing on an auxin-containing medium, it inhibited fungus growth. This inhibition increased with concentration. A decrease in aecialike bodies was observed in all cases wherein GA was a component of the medium. Singly, kinetin (0.01-1.0 mg/liter) had no observable effect on the growth nor morphology of either healthy or infected tissues.

TABLE 1. Volume growth after 90 days of uninfected tissue cultures of *Pinus monticola* and relative growth of *Cronartium ribicola* on infected *P. monticola* tissue cultures induced by auxin-containing media

| Auxin concn mg/l | Indoleacetic acid | | Napthaleneacetic acid | | 2,4-Dichlorophenoxyacetic acid | |
|---|---|---|---|---|---|---|
| | $mm^{3a}$ | Mycelial density[b] | $mm^{3a}$ | Mycelial density[b] | $mm^{3a}$ | Mycelial density[b] |
| 0.00 | 0 | 1 | 0 | 1 | 0 | 1 |
| 0.01 | | | 70 | 1 | 216 | 2 |
| 0.1 | 283 | 1 | 346 | 1 | 690 | 4 |
| 1.0 | 600 | 4 | 632 | 4 | 670 | 6 |
| 3.0 | 612 | 5 | | | 548 | 1 |
| 5.0 | | | 630 | 6 | | |
| 10.0 | 640 | 6 | 651 | 6 | 252 | 1 |

[a] Average of 25-40 cultures.

[b] Mycelial density designated by arbitrary numerical values 1-6, where 1 = a trace of mycelium, 5 = 100% of the tissue culture surface covered by visible mycelium, and 6 = extensive penetration of the medium in addition to the characteristics cited for 5. Other numerals (2, 3, 4) = percentage (25, 50, 75%, respectively) of tissue culture surface covered by visible mycelium.

*Auxin-kinetin effects.*—In combination with any of the effective auxins (IAA, NAA, 2,4-D), kinetin did not influence growth rate; it did enhance vigor and longevity of both host and parasite. This effect was optimum when kinetin was used in a 1/1 to 1/10 ratio with the optimum concentration of each respective auxin.

*IAA-kinetin-gibberellin effects.*—The effects of IAA, kinetin, and GA, in all combinations of concentrations at 0.0, 0.1, 1.0, and 10.0 mg/liter, were tested in the basal medium containing the following additives in mg/liter: vitamin free casein hydrolysate, 100; inositol, 10; ascorbic acid, 0.1; thiamine, 1.0; biotin, 0.01; and pyrodoxine, 0.5. The resultant growth patterns of both healthy and infected explants were identical to those previously described for IAA, kinetin, or GA alone, or for IAA and kinetin in combined concentration ratios. By superimposing GA on the regulator complex, we produced only the inhibitory effect (previously described for GA alone) on the fungus.

*Kinetin-glucose effects.*—Several concentrations of glucose (1, 2, 3, 3.5, and 5%) and kinetin (0.01, 0.1, 1.0, and 10.0 mg/liter) were combined in the basal medium, plus 0.01 mg/liter 2,4-D, to ascertain the validity of a previously observed relationship between these compounds and the formation of a bright-red chromophore in the surface cells of healthy and infected tissue cultures. High concentrations of kinetin (in excess of 1.0 mg/liter) in combination with high concentrations of glucose (in excess of 1.0%) resulted in heavy, localized deposits of the red pigment. Along with this pigment formation, growth of both healthy and infected tissue cultures and of the rust fungus was reduced. Pigment formation did not occur when either glucose or kinetin was used alone, regardless of concentrations. At low concentrations of kinetin (0.01-0.1), glucose to 3.5% was tolerated without pigment formation or adverse growth effects. These effects were also observable when using IAA or NAA (1.0 mg/liter) as an auxin source. In the absence of auxin, the onset of tissue necrosis masked results.

DISCUSSION.—An exogenous supply of any one of the three effective auxins (IAA, NAA, 2,4-D), in adequate concentrations, was the only absolute requirement for inducing both callus initiation and growth of either healthy or blister rust-infected cortex explants of western white pine. Higher concentrations of these auxins provided an additional benefit; they induced massive proliferation of rust mycelia and apparent attempts to sporulate. The inhibitory effect on the blister rust fungus by 2,4-D (3 mg/liter) is interesting in that host tissues do not exhibit a toxic response at this level. Therefore, mycelial growth of this rust can be encouraged or inhibited by the proper selection of auxin and concentration levels. The inhibitory effect of GA was similar to that of 3 mg/liter 2,4-D, but occurred at all concentrations tested. These effects are presumed to have been induced by changes in host metabolism.

The beneficial effects of kinetin were apparent only when it was used in combination with an auxin. These benefits were apparent in both the rust and the host. Again, effects on the rust are presumed to have been derived from changes in supporting host tissues.

It was hoped that experimentation with the auxin-kinetin and auxin-kinetin-gibberellin ratios would induce morphological changes in host tissues and/or duplicate the early spring physiological state that normally results in the production of aeciospores by the blister rust fungus in vivo. Since this experimentation did not result in changes in host morphology nor in the production of aeciospores by the rust fungus, it appears that these processes are not completely controlled by these substances and/or their interactions.

The localized red pigment formation induced by high kinetin-sugar levels is microscopically identical to that formed in response to localized invasions by mycelia of the blister rust fungus. This pigment apparently is formed when host cells are undergoing a specific type of physiological stress. This may indicate that the rust fungus is affecting, directly or indirectly, the kinetin and/or carbohydrate levels in infected tissues.

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