Phytochemistry, Vol. 25, No. 3, pp. 611–612, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
© 1986 Pergamon Press Ltd.

BIOTIC ELICITORS OF DEFENSE REACTIONS IN LODGEPOLE PINE

R. H. MILLER, A. A. BERRYMAN* and C. A. RYAN†

Rangeland Insect Laboratory, Montana State University, Bozeman, MT 59717, U.S.A.; *Department of Entomology, Washington State University, Pullman, WA 99164, U.S.A.; †Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, U.S.A.

(*Revised received* 10 *September* 1985)

Key Word Index—*Pinus contorta*; Pinaceae; lodgepole pine; *Dendroctonus ponderosae*; monoterpenes; biotic elicitors; pectic fragments; chitosan; induced plant defense.

Abstract—Elevated levels of defensive chemicals (monoterpenes) were detected in lodgepole pine (*Pinus contorta* var. *latifolia*) phloem surrounding sites inoculated with living mountain pine beetles (*Dendroctonus ponderosae*), a blue-staining fungus (*Ceratocystis clavigerum*), a pectic fragment from tomato leaves (PIIF) and a fungal cell wall fragment (chitosan). Chitosan elicited the greatest production of monoterpenes at the lowest concentrations, and also elicited greater responses in large, fast-growing trees. Chitosan may prove to be a useful material for assaying the resistance of conifers to lethal bark beetle attacks. The results suggest a common recognition-defense mechanism in higher plants.

INTRODUCTION

A number of bark beetles (Coleoptera: Scolytidae) attacking living conifers induce a complex series of physiological reactions in their host, including the accumulation of terpenoid and phenolic compounds in the tissues surrounding the attack site [1–3]. These dynamic defenses of conifers are similar, in many ways, to the hypersensitive responses of non-woody plants [4–8]. Plant and fungal cell-wall fragments are known to stimulate the production of defensive chemicals in certain legumes and solanaceous plants [9] and one of these, the so-called proteinase inhibitor inducing factor or PIIF, is a pectic fragment isolated from tomato leaves [10]. Another is chitosan, a β-1,4-glucosamine polymer derived from fungal cell walls [11]. We wished to see if these chemicals would elicit defensive reactions in a conifer. We chose lodgepole pine, *Pinus contorta* Douglas var. *latifolia* Engelmann, because of our familiarity with its defense against the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and associated fungi, notably *Ceratocystis clavigerum* (Robinson et Davidson) [2, 12].

RESULTS AND DISCUSSION

Preliminary trials were conducted in 1983 in lodgepole stands on the Colville National Forest (Kettle Falls Ranger District). Two female mountain pine beetles were caged at breast height on opposite sides of five lodgepole pines. Each pine was then inoculated nearby with a blue-staining fungus carried by the beetle. The fungus was obtained from culture No. Colo. 453, USDA Forest Service Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, and was inoculated into holes bored into the tree with a 5 mm cork borer [13]. At the same time, 0.1 ml of a solution of 2 mg/ml of either PIIF [10] or chitosan [11] in a 50 mM phosphate buffer (pH 6.5) was also injected into similar holes in each pine. Treatments were assigned to random locations on the circumference of the tree at breast height. After 3 days the phloem tissue surrounding the inoculation site was excised and frozen prior to chemical analysis. Monoterpene composition of the phloem was determined by gas-chromatography [12], sugars by the anthrone test and starches by the method of Raabo and Tirkildsen [14]. The results of these preliminary tests demonstrated that monoterpene synthesis was induced, probably from sugars, by all four treatments and that the greatest response was elicited by chitosan (Table 1).

The experiment was repeated in 1984 in two lodgepole pine stands on the St. Joe National Forest (Potlatch Ranger District). Five trees in each stand were inoculated as before with *C. clavigerum*, PIIF and chitosan. The two chemicals were introduced at concentrations of 0.01, 0.10, 1.00 and 2.00 mg/ml in 0.1 ml 50 mM phosphate buffer. Analysis of monoterpene concentrations in the tissues surrounding the inoculations 3 days after treatment showed elevated concentrations ($\times 3$) in the treatments (Fig. 1). Although chitosan usually elicited somewhat higher monoterpene synthesis, particularly at the lowest concentration tested, they were not significantly greater than those resulting from PIIF or fungus inoculation (Fig. 1).

It appears that inoculation with chitosan or *C. clavigerum* can be used to detect differences in the defensive ability of lodgepole pine stands (Fig. 1). Chitosan and the fungus elicited consistently higher monoterpene concentrations in stand A, a larger diameter, faster growing stand. This suggests that chitosan may prove to be a useful test for assaying lodgepole pine stand resistance to mountain pine beetle infestation.

Finally, the demonstration that plants in the Pinaceae respond to the same fragments of plant and fungal cell walls that elicit wound reactions in Solanaceae and Leguminosae suggests that a common mechanism of recognition for induced defense may be present in all higher plants.

Acknowledgements—Scientific Paper No. 7152, College of Agriculture and Home Economics Research Center, Washington

Table 1. One-way analysis of variance and *a posteriori* test for differences among mean titres of total monoterpenes and carbohydrates (each reported as mg/g dried phloem) in lodgepole pine phloem surrounding sites inoculated with living *Dendroctonus ponderosae*, *Ceratocystis clavigerum*, PIIF and chitosan (see text) (Kettle Falls Ranger District, Colville National Forest, 1983)

	Inoculum*				
	None	*D. ponderosae*	*C. clavigerum*	PIIF	Chitosan
Monoterpenes	2.4[a]	9.2[b]	7.4[b]	8.4[b]	21.3[c]
Soluble sugars	67.8[a]	53.9[b]	58.9[b]	59.9[b]	53.8[b]
Starch	12.5[a]	15.2[b]	15.5[b]	14.5[b]	13.5[c]

* Means within a horizontal row, followed by the same letter are not significantly different ($P < 0.05$; Duncan's multiple range test).

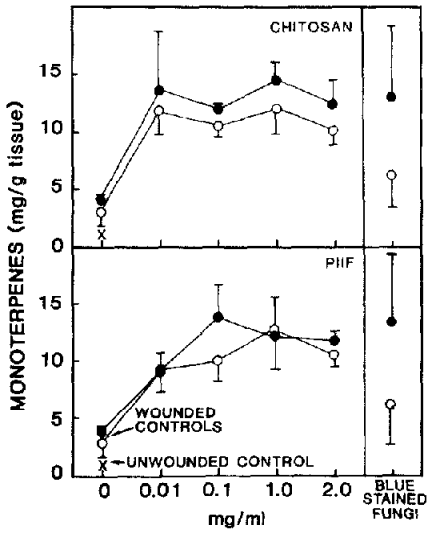

Fig. 1. Monoterpene concentrations in phloem tissue surrounding the site of inoculation with various concentrations of chitosan (upper graph) and PIIF (lower graph), as compared to wounds infected by *C. clavigerum* (fungi), and uninfected wounds in two stands (stand A, solid data points; stand B, open data points).

State University. This project was supported by the National Science Foundation Grants PCM 8203285 and PCM 8315427, and by the Science and Education Administration of the U.S. Department of Agriculture under Grant 82-CRCR-1-1101 and was conducted as part of Regional Project W-110. We thank Dr. Lee Hadwiger of Washington State University for the chitosan.

REFERENCES

1. Berryman, A. A. (1969) *Can. Entomol.* **101**, 1033.
2. Reid, R. W., Whitney, H. S. and Watson, J. A. (1967) *Can. J. Botany* **45**, 1115.
3. Wong, B. L. and Berryman, A. A. (1977) *Can. J. Botany* **55**, 2358.
4. Berryman, A. A. (1972) *BioScience* **22**, 598.
5. Goodman, R. N. (1983) in *Plant Disease* (Horsfall, J. S. and Cowling, E. B., eds). Academic Press, New York.
6. Kuc, J. (1982) *BioScience* **32**, 854.
7. Sequeira, L. (1979) in *Recognition and Specificity of Plant–Host Parasite Interactions* (Daly, J. M. and Vsiting, I., eds). Academic Press, New York.
8. West, C. A. (1981) *Naturwissenschaften* **68**, 447.
9. Ryan, C. A. (1984) in *Advances in Plant Gene Research* (Verma, D. S. P. and Hohns, T., eds) pp. 321–332. Springer, Berlin.
10. Bishop, P. D., Pearce, G., Bryant, J. and Ryan, C. A. (1984) *J. Biol. Chem.* **259**, 13172.
11. Hadwiger, L. and Beckman, J. M. (1980) *Plant Physiol.* **66**, 205.
12. Raffa, K. F. and Berryman, A. A. (1982) *Environ. Entomol.* **11**, 486.
13. Wright, E. (1933) *Phytopathology* **23**, 487.
14. Raabo, E. and T. C. Tirkildsen (1960) in *The Enzymatic Determination of Glucose in Whole Blood, Plasma, or Serum at* 435–476 *nm*. Sigma Tech. Bull., No. 510.