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PIPERIDINE ALKALOIDS IN NITROGEN FERTILIZED *Pinus ponderosa*

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Abstract—We fertilized individual, pole-size ponderosa pine trees at two low-quality sites and pine saplings at a relatively high-quality site, with ammonium nitrate. Six to 12 months later, we measured total %N and 2,6-disubstituted piperidine alkaloids in the foliage. The N additions raised foliar %N above deficiency levels (i.e., from 1.0–1.1% to 1.4–1.6%) at the low-quality sites, but did not elevate foliar %N in saplings at the higher quality site, where it was already (1.9%) well above critical levels. In control trees with foliar N below a threshold of 1.1%, we detected no more than trace levels of alkaloids, indicating that alkaloid production is highly constrained by N deficiency. The N additions increased mean concentrations of the predominant alkaloid, pinidine, at all three sites. Mean total alkaloid concentrations for fertilized trees at the two low-quality sites were 12 and 155 μg/g dry wt higher than controls (relative increases of 12× and 4.5×, respectively). For saplings at the high-quality site, the mean total increased by 584 μg/g dry wt (1.6×) with the N additions. Allocation of foliar N to alkaloids was highest in fertilized saplings (0.81%) compared to control saplings (0.53%). These findings demonstrate that foliar alkaloid concentrations can be increased by nitrogen fertilization of forest trees growing on both low- and high-quality sites. Fertilizing for the purpose of inhibiting potential herbivores may be more successful at higher quality sites where alkaloid levels are enhanced relative to food quality (foliar %N).

Key Words—2,6-Disubstituted piperidine alkaloids, *Pinus ponderosa*, ponderosa pine, fertilization, nitrogen availability, foliar nitrogen, Pinaceae.

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INTRODUCTION

Ponderosa pines, *Pinus ponderosa* Dougl. ex Laws., have *cis*-2,6-disubstituted piperidine alkaloids in their foliage (Tallent et al., 1955; Stermitz et al., 1994), which may contribute to chemical defense against herbivores or pathogens (Schneider et al., 1991; Tawara et al., 1993; Stermitz et al., 1994; Kamm et al., 1998). Concentrations of the Pinaceae alkaloids vary qualitatively and quantitatively within and among sites (Tawara, 1994; Todd, 1994; Tawara et al., 1995; Todd et al., 1995; Gerson and Kelsey, 1998), but the extent to which these alkaloid profiles are environmentally controlled is poorly understood. Observational field studies have shown positive correlations between nutrient availability and alkaloid levels in trees (Gerson and Kelsey, 1998; Hoft et al., 1998), but a causal relationship could only be suggested by these studies. Controlled experiments have determined that nutrients, light, water, and herbivory can interact to influence foliar alkaloid levels in a variety of plants (e.g., Johnson et al., 1987; Krejsa et al., 1987; Baldwin and Ohnmeiss, 1994; Hoft et al., 1996; Ralphs et al., 1998), but such relationships have not been established for conifers.

Of the environmental variables known to influence alkaloids, nutrients are most readily controlled in the silviculture of pines. Light levels can be altered by thinning, but concurrent effects on water and nutrient levels would confound the light treatments. Fertilizing pine forests has been shown to increase foliar concentrations of other secondary metabolites (Bjorkman et al., 1991; McCullough and Kulman, 1991), but effects on herbivores have been mixed (e.g., Smirnoff and Bernier, 1973; Bjorkman et al., 1991; McCullough and Kulman, 1991; Wickman et al., 1996). Ponderosa pine is widely distributed in the western United States, often growing in harsh environments where soil fertility, droughty conditions, and temperature extremes contribute to nutrient limitations. Ponderosa pine forests also are subject to large-scale outbreaks of defoliators (Furniss and Carolin, 1977) that can seriously impact forest values (McMillin and Wagner, 1989). Thus, we were interested in ascertaining whether nitrogen additions could be used to increase foliar alkaloid concentrations under field conditions.

Our previous work with ponderosa pine in central Oregon established that foliar alkaloid levels can vary dramatically among sites (Gerson and Kelsey, 1998). Alkaloid concentrations were strongly correlated with stand density, and foliar nitrogen explained some of the variability, but genetic variation also was thought to contribute to the striking differences in alkaloid concentrations among the three sites. Trees from one site (site A) had virtually no alkaloids. This site carried a densely packed stand of small-diameter trees with 28 m^2/ha basal area on infertile pumice soil. The stand was planted in the 1940s, probably from an offsite seed source, and, therefore, most likely to be genetically distinct from the two naturally regenerated stands. We returned to site A for the current study because we knew these trees were alkaloid deficient and would provide a good

test for our fertilizer treatments. For comparison, we also fertilized individual trees at site C from the previous study, which had alkaloids in every tree sampled. To test the effect of nitrogen additions on ponderosa pine with high alkaloid levels (Gerson and Kelsey, 1999), we randomly fertilized individual saplings in a Willamette Valley, Oregon plantation (hereafter, site D).

METHODS AND MATERIALS

Site Descriptions. Three sites were chosen to represent a range of inherent nitrogen availability and piperidine alkaloid levels in ponderosa pine. The lowest quality site (A) and the intermediate site (C) are located in the Deschutes National Forest (N.F.) in central Oregon and are the same sites previously described in detail (Gerson and Kelsey, 1998). The same site labels (i.e., A and C) are retained here for continuity, although different sample trees were selected. There is no site B designated in this paper. Annual precipitation and site productivity are approximately 38 cm and 3.0 m^3 biomass/ha/yr, respectively, for site A; and 38–43 cm and 3.75 m^3 biomass/ha/yr, respectively, for site C (Bend-Ft. Rock Ranger District, Deschutes N.F., records on file). Foliar %N, measured in August 1996 (Gerson and Kelsey, 1998), averaged 1.0% at site A and 1.3% at site C in current-year foliage; in previous-year foliage, foliar %N averaged 1.2% at site A and 1.5% at site C. Available N in soil was 4.7 μg/g at site A and 18.0 μg/g at site C. Mean total alkaloid concentrations measured in previous-year foliage in April 1996 were 0 μg/g at site A and 33 μg/g at site C. Trees at site A were planted 50–60 years ago; trees at site C were naturally regenerated and approximately the same age.

The highest quality site (D) is located roughly 150 km west of sites A and C at Peavy Arboretum (4945 km N, 482 km E, UTM zone 10; 140 m elevation) in Oregon State University's McDonald Research Forest. Trees at site D were 3- to 4-year-old saplings from a Willamette Valley seed source (zone 262-0.5). Annual precipitation and site productivity are approximately 102 cm and 11.3 m^3 biomass/ha/yr, respectively. We had no prior knowledge of foliar %N or available N in soil at this location, but we knew the trees were likely to have high alkaloid concentrations because a few had been sampled for a methodology study (Gerson and Kelsey, 1999).

Fertilizer Application. At sites A and C, eight pairs of trees were identified based on proximity and attributes (similar diameter, height, crown). One tree from each pair was randomly selected to receive the nitrogen treatment, the other was a control. On May 1, 1997, a broadcast spreader was used to apply nitrogen, phosphorus, and sulfur (or just phosphorus and sulfur for controls) in a 100-m^2 circular plot around the sample trees. The application rates were 224 kg N/ha, 112 kg P/ha, and 34 kg S/ha, as ammonium nitrate (NH_4NO_3), triple super phos-

phate [$Ca(H_2PO_4)_2$], and gypsum ($CaSO_4 \cdot 2H_2O$), respectively (Cochran, 1973). Phosphorus and sulfur were applied, in addition to nitrogen, at sites A and C because of deficiencies in central Oregon pumice soils (Cochran, 1978; Will and Youngberg, 1978). Existing vegetation (bitterbrush, grasses) within the plots was not removed.

At site D, 32 saplings in good condition were identified, and competing vegetation (grasses, bracken fern, poison oak) was controlled with glyphosate and manual removal prior to fertilizer application. On May 12, 1997, sixteen of these saplings were randomly selected to receive the fertilizer treatment: ammonium nitrate at 224 kg N/ha over a 1-m^2 circular plot. Control trees were not provided with any nutrients. Two days later, 4 liters of water was sprinkled over each circular plot to dissolve any remaining fertilizer.

Foliar Sampling and Extraction. At site D, foliage from the current growing season was sampled six months after fertilization (October 27, 1997). Ten fascicles of needles were taken from lateral stems in the top whorl and stored intact in open paper bags for one to two weeks (Gerson and Kelsey, 1999). Ponderosa pine typically produces three needles per fascicle; from each fascicle we allocated two needles for alkaloid extractions and the third to nitrogen analysis. Samples were extracted as described by Gerson and Kelsey (1998), except the needles were air-dried rather than frozen, and a 10-ml aliquot of the supernatant was measured following centrifugation to improve accuracy.

At sites A and C, foliage from the 1997 growing season was sampled 12 months after fertilization (April 21, 1998). In late August 1997, nearly four months after application, some pelletized fertilizer (~5–10%) was still visible on the soil at sites A and C. These sites had received little rainfall since the fertilizer was applied; most annual precipitation in this region occurs as snow. Consequently, we postponed sampling at sites A and C until April 1998 to allow additional time for snowmelt and microbial processes to make the N available to the trees, but not so much time that the pulse of N could be diluted into the 1998 foliar growth. Two branch tips from the north aspect and two from the south were clipped from the middle third of the live crown with a pole pruner and stored separately (by aspect) in paper bags. The following day, 10–15 fascicles were taken from each branch, then dried for three days at 70°C. Dried needles were ground to pass a 20-mesh screen, a portion was allocated to N analysis, and the remainder was stored in zip-lock bags at 2–5°C until extraction. Samples from north and south aspects were treated as duplicates for the alkaloid analysis. A slightly modified methodology (Gerson and Kelsey, 1999), developed after the analysis of site D samples, was followed for site A and C samples. The use of chloroform and extra rinsing in the extraction procedure was found to improve alkaloid yields, whereas differences in the sample drying procedures were found to be inconsequential.

Nitrogen Analysis. Composite samples were prepared for each sample tree

at sites A and C by combining equal weights of ground foliage from north and south aspects. Dried needles remaining from site D samples also were ground (20 mesh). Samples were analyzed for total percentage of nitrogen following micro-Kjeldahl digestion by the Plant & Soil Analytical Lab, Department of Forest Science, OSU, by using an Alpkem-RFA 300 rapid-flow colorimetric analyzer.

Alkaloid Analysis. All known alkaloids in the foliar extracts were quantified by GC-MS as previously described (Gerson and Kelsey, 1998). These compounds were identified according to published mass spectra (Hart et al., 1967; Tawara et al., 1993) as pinidine [2-methyl-6-(2-propenyl)piperidine], pinidinol [2-methyl-6-(2-hydroxypropyl)piperidine], 1,2-dehydropinidinol [2-methyl-6-(2-hydroxypropyl)-1,2-piperideine], pinidinone [2-methyl-6-(2-oxopropyl)piperidine], 1,2-dehydropinidinone [2-methyl-6-(2-oxopropyl)-1,2-piperideine], and euphococcinine (1-methyl-9-nor-3-granatanone).

Statistical Analysis. Data from sites A and C were analyzed separately from site D because of differences in tree age, sampling, and alkaloid extraction procedures. Fertilizer effects on total N and alkaloid concentrations in foliage were tested using paired, one-tailed *t* tests for sites A and C, and unpaired, one-tailed *t* tests for site D (SAS Institute, Inc., 1989).

RESULTS AND DISCUSSION

Foliar Nitrogen. As expected from our previous work (Gerson and Kelsey, 1998), total foliar N (% dry weight basis) was lowest in controls at site A and slightly higher in site C controls (Table 1), confirming that trees at site A had the least favorable nutrient status prior to fertilization. Nitrogen deficiencies in conifers may be identified by foliar %N below a critical level required for growth. Compared to other conifers, the critical level of foliar N is low for ponderosa pine: approximately 0.9% (Oliver and Ryker, 1990) to 1.1% (Powers et al., 1988) for pole-sized trees. Nitrogen concentrations in current-year foliage of all control trees at site A fell below 1.1%, indicating these trees were N deficient. Control trees at site C had borderline deficiency levels, ranging from 1.0 to 1.2%. Not surprisingly, N fertilization increased total N in foliage at sites A and C: fertilized trees had 0.3–0.9% higher foliar N at site A, and 0.1–0.6% higher foliar N at site C, compared to controls at each site. In a similar study, mature ponderosa pine with low foliar N levels responded similarly to ammonium nitrate fertilization within one year (Zabowski and Henry, 1995).

At site D, the range of foliar N in both control and fertilized saplings (1.6–2.1%; Figure 1) was entirely above the range measured in the central Oregon trees, and the fertilizer treatment did not increase total N in the current-year foliage (Table 1). In another study, field fertilization of ponderosa pine seedlings growing at a nutrient-rich site also had no effect on foliar N concentrations (in the

TABLE 1. TOTAL NITROGEN CONCENTRATION IN FOLIAGE

Site	N	Concentration (% dry wt, mean ± SE)		Prob > |t|
		Control	N-fertilized	
A	8	0.99 ± 0.07	1.55 ± 0.15	<0.001[a]
C	8	1.10 ± 0.06	1.41 ± 0.13	0.001[a]
D	16	1.89 ± 0.10	1.93 ± 0.10	0.133[b]

[a]Paired, one-tail *t* test.
[b]One-tail *t* test.

1.6–1.9% range) or on growth of the seedlings (Gleason et al., 1990). Growth responses to the fertilizer were not measured in our study, except for needle lengths of samples from site D. We found no difference in needle length between the treatments (data not shown). From the above, we conclude that foliar N in sample trees at site D was probably near optimal.

Alkaloid Response to N Additions. Pinidine, the primary end product of piperidine biosynthesis in ponderosa pine (Tawara et al., 1993), is the most ubiquitous and abundant of the *Pinus* alkaloids in foliage from the study areas (Gerson and Kelsey, 1998, 1999). Therefore, N effects on pinidine are of particular interest. The nitrogen treatments enhanced pinidine concentrations at all

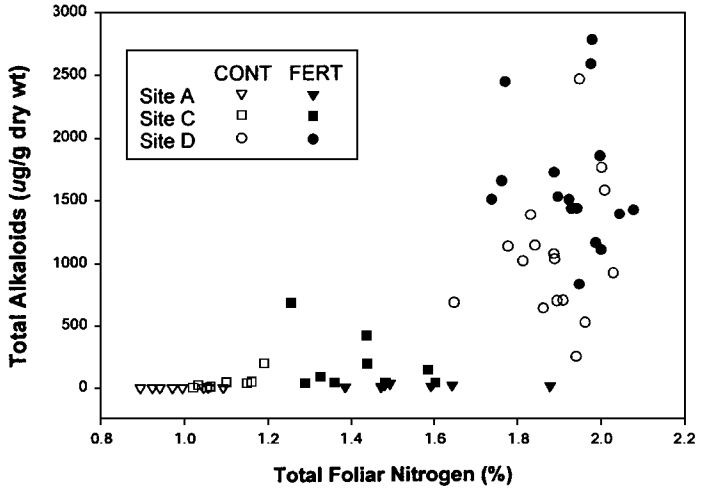

FIG. 1. Total alkaloid concentrations relative to total percentage of nitrogen in foliage. N = 8 control and 8 nitrogen fertilized trees at sites A and C; N = 16 control and 16 nitrogen fertilized saplings at site D.

TABLE 2. PIPERIDINE ALKALOIDS IN FOLIAGE AT SITES A AND C IN CENTRAL OREGON

Alkaloid	Concentration (μg/g dry wt, mean ± SE)[a]		Prob > $\|t\|$[b]
	Controls	N-fertilized	
Site A			
Pinidine	0 ± 0	12 ± 6	0.039
Other piperidines	0 ± 0	0 ± 0	0.999
Total	0 ± 0	12 ± 6	0.039
Site C			
Pinidine	44 ± 23	175 ± 67	0.015
1,2-Dehydropinidinol	0 ± 0	19 ± 13	0.096
Pinidinol	0 ± 0	0 ± 0	0.999
1,2-Dehydropinidinone	0 ± 0	2 ± 2	0.175
Pinidinone	0 ± 0	0 ± 0	0.999
Euphococcinine	0 ± 0	2 ± 2	0.175
Total	44 ± 23	199 ± 83	0.022

[a] $N = 8$.
[b] Paired t tests.

three sites. At site A, where practically no piperidine alkaloids were detected in our previous study, four of the eight nitrogen-fertilized trees produced 10–44 μg/g dry wt of pinidine, and none of the control trees produced any pinidine. The pinidine response was not large (Table 2), but it is meaningful in that it demonstrates the trees at this site are not absolutely (genetically) constrained from producing the *Pinus* alkaloids. A similar response (but at higher alkaloid levels) was observed at site C (Table 2). Among the eight pairs of trees, the N-fertilized tree had 9–353 μg/g dry wt more pinidine after 12 months. At site D, all 32 trees contained pinidine in concentrations ranging from 204 to 1071 μg/g in controls, and from 375 to 1235 μg/g in fertilized trees, yet the mean concentration of pinidine was significantly higher for the fertilized trees (Table 3). The high variation in alkaloid concentrations within sites was typical (Gerson and Kelsey, 1998).

Nitrogen effects varied for the other *Pinus* alkaloids, although generally the effect was stronger where alkaloids were present in higher concentrations (Tables 2 and 3). None of the *Pinus* alkaloids, other than pinidine, was detected in any of the sample trees at site A. At site C, small concentrations (15–100 μg/g dry wt) of the 1,2-piperideines and euphococcinine were found only in the two N fertilized trees with the most pinidine. Therefore, the additional N had only a marginal effect at best (all $P \geq 0.096$), on concentrations of alkaloids other than pinidine at sites A and C. At site D, most of the *Pinus* alkaloids were present in control and fertilized trees. A nitrogen effect was clear for 1,2-dehydropinidinol (Table 3), which had concentrations comparable to pinidine. Interestingly,

TABLE 3. PIPERIDINE ALKALOIDS IN FOLIAGE AT SITE D IN WILLAMETTE VALLEY

Alkaloid	Concentration (μg/g dry wt, mean ± SE)[a]		Prob > $\|t\|$[b]
	Controls	N-fertilized	
Pinidine	598 ± 59	775 ± 63	0.026
1,2-Dehydropinidinol	316 ± 84	639 ± 105	0.011
Pinidinol	42 ± 12	87 ± 12	0.056
1,2-Dehydropinidinone	58 ± 13	85 ± 17	0.105
Pinidinone	3 ± 2	8 ± 3	0.066
Euphococcinine	39 ± 5	45 ± 4	0.156
Total	1055 ± 542	1639 ± 537	0.002

[a] $N = 16$.
[b] One-tail t tests, with equal/unequal variance as appropriate.

the proportion of 1,2-dehydropinidinol in the total alkaloid sum increased by about 10%, and pinidine decreased correspondingly, in the N-fertilized saplings. Concentrations of the remaining alkaloids were below 100 μg/g dry wt, and although means were higher in all cases for the N fertilized trees, the differences were only marginally significant (all $P \geq 0.056$). The 1,2-piperideines, pinidinol, and pinidinone are thought to be biosynthetic precursors to pinidine (Leete and Juneau, 1969; Leete et al., 1975; Tawara et al., 1993, 1995), and euphococcinine is probably an alternative end product (Tawara et al., 1993), so N effects on the concentrations of intermediate compounds may have been less apparent because of their transitory nature. When cumulative sums of the alkaloids are evaluated, the N effect becomes more apparent. Total alkaloid concentrations were increased by the N additions at all three study sites (Tables 2 and 3). At site D especially, the cumulative effect was an order of magnitude stronger than effects on any individual alkaloid.

The relationship between foliar N and alkaloid levels (Figure 1) revealed that allocation of N to constitutive alkaloids is partially dependent on availability. No more than trace levels of alkaloids were observed in trees at sites A or C with <1.1% foliar N, demonstrating that alkaloid production in ponderosa pine is highly constrained when foliar N is deficient. Alkaloid synthesis in fertilized trees at site A was low in comparison to site C, relative to the increase in foliar %N at both sites, indicating that factors other than N availability also played a role. Alkaloid biosynthesis is dependent on enzyme systems that may be diminished in plants grown in nutrient-poor conditions (Johnson et al., 1989). If alkaloids accumulate early in developing leaves and are diluted with age (Ralphs et al., 1998), which appears to be the case for ponderosa pine (Tawara et al., 1995; Gerson and Kelsey, 1998), then the timing of nitrogen fertilization, relative to tree phenology, could limit the alkaloid response to N additions, even though foliar %N increases. Trees at sites A and C were phenologically similar

TABLE 4. PROPORTION OF LEAF NITROGEN ALLOCATED TO ALKALOIDS

Site	*N*	Alkaloid N/Total N (%, mean ± SE)	
		Control	N-fertilized
A	8	0.00 ± 0.00	0.01 ± 0.00
C	8	0.04 ± 0.02	0.14 ± 0.06
D	16	0.53 ± 0.09	0.81 ± 0.09

and fertilized on the same day, but N uptake could have been delayed at site A by the closed canopy or other environmental factors. Alternatively, the trees at site A may simply have been inherently low alkaloid producers (as in Krejsa et al., 1987).

At site D, where foliar N was well above deficiency levels (>1.8%), the N additions had no effect on foliar %N, and there was no correlation between foliar N and alkaloid levels (Figure 1). This apparent decoupling of alkaloid and foliar N levels could result from maximized leaf N (protein) levels and allocation of N to other tissues. The proportion of foliar N in piperidine alkaloids is so small it seems unlikely these alkaloids function as N storage compounds. Increases in alkaloid concentrations at site D, in response to what could be considered excessive N additions, differed from the response of monoterpene indole alkaloids produced by an evergreen broad-leaved tree, *Tabernaemontana pachysiphon* Stapf (Apocynaceae) (Hoft et al., 1996). By using the proportion of foliar N fixed in alkaloids as an index of N allocation, Hoft et al. (1996) noted that moderately and strongly fertilized seedlings allocated less N to alkaloids (48%) than unfertilized seedlings (0.66%). In contrast, fertilized saplings at our site D allocated 0.80% of their foliar N to piperidine alkaloids, whereas 0.53% of foliar N in control trees was incorporated in alkaloids (Table 4). Both studies used young, evergreen, woody plants; however, the involvement of different biosynthetic pathways could account for the different N allocation strategies and alkaloid responses observed for the two species. The *Pinus* alkaloids are synthesized from polyketide precursors (Leete and Juneau, 1969; Leete et al., 1975), whereas the alkaloids of *T. pachysiphon* are from amino acid/monoterpene origins.

Considering the alkaloid responses to N fertilization across the range of initial (site) conditions (Figure 1), it is apparent that foliar N is a meaningful correlate for alkaloid levels under limited circumstances (perhaps for coarse-scale, but not fine-scale comparisons) and that factors other than N availability influence alkaloid levels in ponderosa pine foliage. Mature trees at sites A and C allocated a very small percentage of their foliar N to alkaloids even when leaf N was sufficient, compared to saplings at site D (Table 4). Tree age is a potential factor, in addition to the environmental and genetic differences already discussed, that could have contributed to the variation between the central Oregon

and Willamette Valley sites. Young trees can have N allocation strategies different from older trees (Kozlowski and Pallardy, 1997). For example, protein (and procyanidin) can increase in ponderosa pine seedlings and decrease in pole-size trees in response to defoliation (Wagner, 1988). Hoft et al. (1998) found slightly lower indole alkaloid levels in older *T. pachysiphon* trees. Our data are consistent with the possibility that younger ponderosa pine allocate more foliar N to alkaloids. Piperidine alkaloids accumulate early in seedling growth (Tawara, 1994; Todd, 1994; Tawara et al., 1995; Todd et al., 1995). There have been no controlled comparison of piperidines among young and mature conifers, however. In fact, information regarding the effects of nutrient additions of N-based secondary metabolites comes primarily from greenhouse studies of young plants, and allocation of N to alkaloids in mature woody plants is relatively undocumented.

As noted in our previous study (Gerson and Kelsey, 1998), light herbivory on ponderosa pine does not appear to induce piperidine alkaloid production in existing foliage. Some herbivory was noted on the samples collected at sites A and C in central Oregon, where an outbreak of the pandora moth (*Coloradia pandora* Blake) had recently subsided. Several larvae of this pine defoliator were found in foliage samples from site C. The larval stage of *C. pandora* occurs biennially and was out of phase when we fertilized in May 1997, but larvae emerged the following September, overwintered on the foliage, and fed as temperatures allowed until June 1998. The branches sampled in April 1998 for fertilizer effects were examined for defoliation and assigned an herbivory index denoting the extent of damage. Regressions of these herbivory indices on total alkaloid concentrations at each site indicated no relationship between the variables and confirmed our previous observations. In central Oregon, *C. pandora* larvae complete their feeding stage about the same time that new needles begin elongating, and it remains possible that alkaloid levels in the new foliage could be influenced by herbivory on the older foliage.

Effects of the *Pinus* alkaloids on *C. pandora* larvae have not been tested directly, but fertilization of ponderosa pine in another central Oregon study (Wickman et al., 1996) increased foliar N levels to 2.1%, reduced *C. pandora* larval weights approximately 30%, and increased N in larval frass. These effects on the *C. pandora* larvae may be explained by fertilizer-induced increases in foliar alkaloids and their subsequent excretion [as in Kamm et al., 1998, for western spruce budworm, *Choristoneura occidentalis* (Freeman)]. Fertilizing forests for the purpose of inhibiting herbivory should be weighed against potential benefits to herbivores in terms of food quality (McClure, 1991; Mason et al., 1992). The fertilizer response at site D in our study, where high foliar N levels were not increased while foliar alkaloid levels were, is particularly interesting in this respect. Assuming that %N adequately reflects nutritional quality of the foliage, we can infer that N fertilization to inhibit herbivory of ponderosa pine would

be more effective where nitrogen sufficiency is established (e.g., at high nutrient sites or with repeated fertilizer applications). This inference depends on a quantitative sensitivity of the herbivore to the piperidines, which remains to be demonstrated. Preliminary studies of antifeedant activity by the Pinaceae alkaloids toward lepidopteran larvae (Schneider et al., 1991; Stermitz et al., 1994) have had mixed results (F. R. Stermitz, personal communication).

SUMMARY AND CONCLUSION

Nitrogen fertilization increased total alkaloid concentrations in ponderosa pine foliage at two low quality sites and also at a high quality site. Fertilization increased total N in foliage at the low-quality sites, but not at the high quality site. This study demonstrated that fertilization of ponderosa pine, and by extension, other silvicultural treatments that increase N availability (such as thinning overly dense stands), might be used to increase alkaloid concentrations in foliage. If forthcoming antifeedant research indicates that promoting alkaloid production in pine forests would be desirable, our findings suggest that fertilizing trees that already have high foliar N would likely be more successful in deterring herbivory because alkaloid levels would increase without a concomitant increase in foliar nutritional quality.

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REFERENCES

BALDWIN, I. T., and OHNMEISS, T. E. 1994. Coordination of photosynthetic and alkaloidal responses to damage in uninducible and inducible *Nicotiana sylvestris*. *Ecology* 75:1003–1014.

BJORKMAN, C., LARSSON, S., and GREF, R. 1991. Effects of nitrogen fertilization on pine needle chemistry and sawfly performance. *Oecologia* 86:202–209.

COCHRAN, P. H. 1973. Response of individual ponderosa pine trees to fertilization. USDA Forest Service, Research Note PNW-206, 15 pp.

COCHRAN, P. H. 1978. Response of a pole-size ponderosa pine stand to nitrogen, phosphorus, and sulfur. USDA Forest Service, Research Note PNW-319, 8 pp.

FURNISS, R. L., and CAROLIN, V. M. 1977. Western Forest Insects. USDA Forest Service, Miscellaneous Publication No. 1339.

GERSON, E. A., and KELSEY, R. G. 1998. Variation of piperidine alkaloids in ponderosa (*Pinus ponderosa*) and lodgepole pine (*P. contorta*) foliage from central Oregon. *J. Chem. Ecol.* 24:815–827.

GERSON, E. A., and KELSEY, R. G. 1999. Foliar storage and extraction methods for quantitative

analysis of piperidine alkaloids from ponderosa pine (*Pinus ponderosa*). *Phytochem. Anal.* 10:1–6.

GLEASON, J. F., DURYEA, M., ROSE, R., and ATKINSON, M. 1990. Nursery and field fertilization of 2 + 0 ponderosa pine seedlings: The effect on morphology, physiology, and field performance. *Can. J. For. Res.* 20:1766–1772.

HART, N. K., JOHNS, S. R., and LAMBERTON, J. A. 1967. (+)-9-Aza-1-methylbicyclo[3,3,1]nonan-3-one, a new alkaloid from *Euphorbia atoto* Forst. *Aust. J. Chem.* 20:561–563.

HOFT, M., VERPOORTE, R., and BECK, E. 1996. Growth and alkaloid contents in leaves of *Tabernaemontana pachysiphon* Stapf (Apocynaceae) as influenced by light intensity, water and nutrient supply. *Oecologia* 107:160–169.

HOFT, M., VERPOORTE R., and BECK, E. 1998. Leaf alkaloid contents of *Tabernaemontana pachysiphon* as influenced by endogenous and environmental factors in the natural habitat. *Planta Med.* 64:148–152.

JOHNSON, N. D., LIU, B., and BENTLEY, B. L. 1987. The effects of nitrogen fixation, soil nitrate, and defoliation on the growth, alkaloids, and nitrogen levels of *Lupinus succulentus* (Fabaceae). *Oecologia* 74:425–431.

JOHNSON, N. D., RIGNEY, L. P., and BENTLEY, B. L. 1989. Short-term induction of alkaloid production in lupines: differences between N_2-fixing and nitrogen-limited plants. *J. Chem. Ecol.* 15:2425–2434.

KAMM, C. D., TAWARA, J. N., and STERMITZ, F. R. 1998. Spruce budworm larval processing of piperidine alkaloids from spruce needles. *J. Chem. Ecol.* 24:1153–1160.

KOZLOWSKI, T. T., and PALLARDY, S. G. 1997. Physiology of Woody Plants. Academic Press, San Diego.

KREJSA, B. B., ROUQUETTE, JR., F. M., HOLT, E. C., CAMP, B. J., and NELSON, L. R. 1987. Alkaloid and nitrate concentrations in pearl millet as influenced by drought stress and fertilization with nitrogen and sulfer. *Agron. J.* 79:266–270.

LEETE, E., and JUNEAU, K. N. 1969. Biosynthesis of pinidine. *J. Am. Chem. Soc.* 91:5614–5618.

LEETE, E., LECHLEITER, J. C., and CARVER, R. A. 1975. Determination of the "starter" acetate unit in the biosynthesis of pinidine. *Tetrahedron Lett.* 44:3779–3782.

MASON, R. R., WICKMAN, B. E., BECKWITH, R. C., and PAUL, H. G. 1992. Thinning and nitrogen fertilization in a grand fir stand infested with western spruce budworm. Part I: Insect response. *For. Sci.* 38:235–251.

MCCLURE, M. S. 1991. Nitrogen fertilization of hemlock increases susceptibility to hemlock woolly adelgid. *J. Arbor.* 17:227–229.

MCCULLOUGH, D. G., and KULMAN, H. M. 1991. Effects of nitrogen fertilization on young jack pine budworm (*Pinus banksiana*) and on its suitability as a host for jack pine budworm (*Choristoneura pinus pinus*) (Lepidoptera: Tortricidae). *Can. J. For. Res.* 21:1447–1458.

MCMILLIN, J. D., and WAGNER, M. R. 1989. Assessing the impacts of foliage-feeding insects on timber and scenic beauty of ponderosa pine: a methodological approach, pp. 51–59, *in* A. Tecle, W. W. Covington, and R. H. Hamre (eds.). Multiresource Management of Ponderosa Pine Forests. USDA Forest Service, General Technical Report RM-185.

OLIVER, W. W., and RYKER, R. A. 1990. *Pinus ponderosa* Dougl. ex Laws., pp. 413–424, *in* R. M. Burns and B. H. Honkala (eds.). Silvics of North America, Vol. 1: Conifers. USDA Forest Service, Agriculture Handbook 654.

POWERS, R. F., WEBSTER, S. R., and COCHRAN, P. H. 1988. Estimating the response of ponderosa pine forests to fertilization, pp. 219–225, *in* W. C. Schmidt (ed.). Future Forests of the Mountain West. USDA Forest Service, General Technical Report INT-243.

RALPHS, M. H., MANNERS, G. D., and GARDNER, D. R. 1998. Influence of light and photosynthesis on alkaloid concentration in larkspur. *J. Chem. Ecol.* 24:167–182.

SAS INSTITUTE, INC. 1989. SAS/STAT User's Guide, Ver. 6, 4th ed., Vol. 2. Cary, North Carolina.

SCHNEIDER, M. J., MONTALI, J. A., HAZEN, D., and STANTON, C. E. 1991. Alkaloids of *Picea*. *J. Nat. Prod.* 54:905–909.

SMIRNOFF, W. A., and BERNIER, B. 1973. Increased mortality of the Swaine jack-pine sawfly, and foliar nitrogen concentrations after urea fertilization. *Can. J. For. Res.* 3:112–121.

STERMITZ, F. R., TAWARA, J. N., BOECKL, M., POMEROY, M., FODERARO, T. A., and TODD, F. G. 1994. Piperidine alkaloid content of *Picea* (spruce) and *Pinus* (pine). *Phytochemistry* 5:951–953.

TALLENT, W. H., STROMBERG, V. L., and HORNING, E. C. 1955. Pinus alkaloids. The alkaloids of *P. sabiana* Dougl. and related species. *J. Am. Chem. Soc.* 77:6361–6364.

TAWARA, J. N. 1994. Chemical analyses of *Picea* and *Pinus* (Pinaceae) & biosynthetic studies on *Pinus ponderosa* alkaloids. PhD dissertation. Colorado State University, Fort Collins.

TAWARA, J. N., BLOKHIN, A., FODERARO, T. A., STERMITZ, F. R., and HOPE, H. 1993. Toxic piperidine alkaloids from pine (*Pinus*) and spruce (*Picea*) trees. New structures and a biosynthetic hypothesis. *J. Org. Chem.* 58:4813–4818.

TAWARA, J. N., STERMITZ, F. R., and BLOKHIN, A. V. 1995. Alkaloids of young ponderosa pine seedlings and late steps in the biosynthesis of pinidine. *Phytochemistry* 39:705–708.

TODD, F. G. 1994. Potentially toxic compounds of *Convolvulacae* and piperidine alkaloids of *Picea*. PhD dissertation. Colorado State University, Fort Collins.

TODD, F. G., STERMITZ, F. R., and BLOHKIN, A. V. 1995. Piperidine alkaloid content of Picea pungens (Colorado blue spruce). *Phytochemistry* 40:401–406.

WAGNER, M. R. 1988. Induced defenses in ponderosa pine against defoliating insects, pp. 141–155, *in* W. J. Mattson, J. Levieux and C. Bernard-Dagan (eds.). Mechanisms of Woody Plant Defenses Against Insects. Springer-Verlag, New York.

WICKMAN, B. E., MASON, R. R., and PAUL, H. G. 1996. Ponderosa pine response to nitrogen fertilization and defoliation by the pandora moth, *Coloradia pandora* Blake, pp. 118–126, *in* W. J. Mattson, P. Niemela and M. Rousi (eds.). Dynamics of Forest Herbivory: Quest for Pattern and Principle, USDA Forest Service, General Technical Report NC-183.

WILL, G. M., and YOUNGBERG, C. T. 1978. Sulfur status of some central Oregon soils. *Soil Sci. Soc. Am. J.* 42:132–134.

ZABOWSKI, D., and HENRY, C. L. 1995. Soil and foliar nitrogen after fertiliser treatment of ponderosa pine. *N.Z. J. For. Sci.* 24:333–343.