Interactive Effects of Ozone and Powdery Mildew on Pea Seedlings

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ABSTRACT

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We assessed the effects of ozone and powdery mildew infection on plant growth, ozone injury, colonization of leaves by the fungus, and pisatin content of pea (*Pisum sativum*) seedlings. Seedlings of cultivars Alaska and Bounty grown in the greenhouse were moved to controlled-environment growth chambers and exposed to ozone concentrations of 0.00, 0.06, and 0.12 $\mu l\ L^{-1}$ for 6 h/day (1 $\mu l\ L^{-1}$ = 1,960 $\mu g\ m^{-3}$ at standard temperature and pressure). Fumigations were performed for 5 days before, after, or both before and after inoculation with conidia of *Erysiphe polygoni* f. sp. *pisi*, the causal agent of pea powdery mildew. Ozone at 0.12 $\mu l\ L^{-1}$ suppressed growth in both cultivars only in the preinoculation fumigation treatment, indicating a protective effect of powdery mildew infection, which by itself did not affect dry weight. Bounty was more resistant to infection by *E. p. pisi* than Alaska and was also less sensitive to foliar ozone injury. Colonization of leaves by the fungus resulted in suppressed foliar ozone injury in both cultivars. Both preinoculation and postinoculation exposure to ozone at 0.12 $\mu l\ L^{-1}$ significantly suppressed fungal leaf colonization. The apparent increase in resistance indicated a plant defense mechanism induced by the ozone exposure. Pisatin, a pea phytoalexin, was not detected in leaf tissue of noninoculated plants that were exposed to ozone, however, indicating that pisatin probably is not involved in this increased resistance.

Additional keywords: air pollution, pollutant-parasite interaction.

Ozone is considered the most economically important air pollutant affecting vegetation in North America; it reduces growth, injures foliage, and suppresses yields (8,19,33). In addition to its direct effects, ozone may also influence plant response to other stresses. Thus, to evaluate the full impact of ozone on vegetation, one must understand the interactions between ozone and other stresses, such as pathogens. For example, ozone predisposes plants to infection by *Botrytis cinerea* by providing infection courts (15,16,34). Plants that are exposed to ozone might be rendered more resistant to a pathogen, however, if the pollutant suppresses the pathogen or decreases the suitability of the plant as a host (13,14).

Powdery mildew of pea (*Pisum sativum* L.), caused by the ascomycete *Erysiphe polygoni* DC. f. sp. *pisi* (syn. *E. pisi* Syd.), suppresses both yield and quality of pods (6,22). The effects of ozone on this disease have not been investigated; however, a study of the effects of ozone on powdery mildew of barley, caused by *E. graminis* f. sp. *hordei,* found that plants exposed to the pollutant had fewer infections but that individual fungal colonies were larger (10).

Phytoalexins may play a role in the interaction among plants, pathogens, and pollutants. Phytoalexins may accumulate after physical injury or treatment with various chemicals (3,7) as well as after exposure to microorganisms (24). Phytoalexin synthesis induced by acute ozone stress has been demonstrated in soybean (12), bean (29), and alfalfa (31). Low ambient ozone concentrations that induce phytoalexins could be significant for certain plant diseases (1,11,14) if ozone increases host resistance to disease.

We examined the effects of ozone and powdery mildew on the growth and physiology of pea seedlings. We also investigated the possible involvement of a phytoalexin in an air pollutant-plant disease interaction; this possibility had been suggested (11,14) but not confirmed.

MATERIALS AND METHODS

Plant culture. Seeds of pea cultivars Alaska (indeterminate vine) (Johnny's Selected Seed, Albion, ME) and Bounty (determinate vine, resistant to powdery mildew) (Rogers Brothers Seed Company, Nappa, ID) were sown in 1-L fiber pots in a peat-vermiculite (1:2, v/v) rooting medium amended with 4.5 kg of a slow-release fertilizer (Osmocote, N-P-K ratio 19-6-12; Grace Sierra Horticultural Products, Milpitas, CA) per cubic meter of medium. Plants were grown in a greenhouse maintained at 28 C during the day and 22 C at night with supplemental lighting (multivapor lamps) to provide a 14-h photoperiod from 0600 to 2000.

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Ten days after planting, seedlings were thinned to one plant per pot. Twenty pots of each cultivar were randomly assigned to each of three controlled-environment chambers (Western Environmental, Napa, CA) maintained at 22±1 C during the day and 18±1 C at night, with relative humidity of 65±5% and a 14-h photoperiod (0600–2000; photosynthetic photon flux density 620±30 μmol $m^{-2}s^{-1}$). The variations in these environmental parameters were the same for each chamber. Airflow of 14 m^3 min^{-1} was maintained in the chambers, which were approximately 8 m^3 in volume. Plants were irrigated with tap water in the mornings before exposures began and were well watered throughout the experiment.

Ozone exposure. Activated carbon filters maintained the ozone concentration in the chambers at less than 0.005 μl L^{-1}. Ozone fumigations began 11 days after seeding when the third true leaf of the seedlings was fully expanded. Ozone was added to two chambers to achieve concentrations of 0.060±0.005 or 0.120±0.005 μl L^{-1}. The third chamber (no ozone added) was the control. Seedlings were exposed for 6 h each day (0900–1500). Ozone was generated by dry-air electrical discharge in an ozone generator (Griffin Technics, Lodi, NJ) supplied with pure oxygen. The pollutant was injected into the inlet airstream of the chambers.

Teflon tubing was used for all air sampling. Ozone concentrations in the chambers were monitored continuously during exposures with either a chemiluminescent ozone analyzer (model 8410, Monitorlabs, San Diego, CA) or an ultraviolet (UV) photometric ozone analyzer (model 49, Thermo Environmental Instruments Inc., Hopkinton, MA), and values were recorded by a strip-chart recorder. Analyzers were calibrated before and after experiments according to the U.S. Environmental Protection Agency standard (5).

Inoculation protocol. Conidia of *E. p. pisi* were obtained from peas grown in a lightroom. The fungus was propagated through inoculation of "disease stock" Alaska pea plants that were kept in a separate lightroom with a 14-h photoperiod. New stock plants were inoculated every 2 wk.

Experimental plants were inoculated in the evening, 16 days after seeding. The "rolling" method (23) was used for inoculations, except that rather than depositing spores from source plants onto a glass slide, we applied the spores (conidiospores) directly to leaves of the target plants. All fully expanded leaves (four true leaves) were inoculated.

Measurements. Heights of plant shoots were measured every other day over the course of the treatment in the environmental chambers, beginning 1 day before the first exposure. Visible foliar ozone injury was assessed on each leaf every 2 days, beginning after the second day of ozone exposure when initial injury symptoms became apparent. To quantify leaf injury, we estimated the percentage of leaf area with mottling, chlorotic or necrotic lesions, or bifacial necrosis (26).

Disease severity was estimated as the percentage of leaf area that was covered by a visible layer of *E. p. pisi* mycelium. Severity was assessed daily once mycelium was macroscopically detectable (usually 3 days after inoculation). Each leaf was assessed separately, and the assessment was performed by the same person in all experiments.

Plants were harvested after the final day of fumigation. The area of each fully expanded leaf (six true leaves per plant at harvesttime) was measured with a portable area meter (model LI-300, Lambda Instruments, Lincoln, NE). Shoots were freeze-dried, dry weights were determined, and leaf samples were analyzed for pisatin content.

Pisatin assay. D. E. Matthews, Department of Biometry and Plant Breeding, Cornell University (*personal communication*), provided a protocol for the extraction of pisatin from pea seed based on previously described methods (29). All leaves of the plants for each treatment (combination of ozone concentration, cultivar, and inoculation) were combined for the pisatin extraction. Lyophilized samples were ground in liquid nitrogen and stored at −18 C. A 2-g subsample of each sample was placed in a 150-ml Erlenmeyer flask, covered with 50 ml of *n*-hexane (HPLC-grade, Fisher Scientific, Springfield, NJ), and shaken overnight. A pure pisatin standard (50 μg) provided by L. Delserone (Department of Plant Pathology, Cornell University) was coextracted to determine the percentage of loss due to the extraction procedure.

The extract was vacuum-filtered through Whatman filter paper (qualitative grade, medium porosity), and the plant material was discarded. The filtrate was evaporated under vacuum, and the residue was dissolved in 6 ml of methanol. Samples were stored at −18 C for 0.5 h and filtered through glass wool (compacted at the bottom of the wide part of a Pasteur pipet) at −18 C. The methanol was evaporated under a nitrogen stream, and the residue was dissolved in 200 μl of 95% ethanol and stored at −18 C. The total ethanol fraction of each sample was loaded onto silica gel preparative layer chromatography plates with a UV-fluorescence indicator (20 × 20 cm, layer thickness 0.5 mm) (PSC-Fertigplatten Kieselgel 60 $F_{254}S$, Artikel 13794, Merck, Darmstadt, Germany), and the plates were developed in a solvent system of chloroform-methanol (25:1). The coextracted pure pisatin sample was cochromatographed with the actual samples.

After chromatography, the plates were air-dried and viewed under a UV lamp (254 nm). The silica gel with the bands that had comigrated with the pisatin standard was removed from the plates and eluted with 2 ml of 95% ethanol. A UV spectrophotometer was used to scan absorbance of the samples between 260 and 320 nm. For the samples that showed the characteristic absorbance scan of pisatin, with a major peak at 309 nm and a shoulder at 286 nm, the A_{309nm} value was used to calculate the concentration c in grams per liter from the formula $c = A \times MW / \epsilon$, where A is absorbance at 309 nm in ethanol, MW is the molecular weight of pisatin (314 g per mole), and ϵ is the molar extinction coefficient of pisatin in ethanol (ϵ = antilog 3.86 = 7,244 [liters per mole]) (27).

Experimental design. Two sets of experiments (A and B) with different ozone exposure regimes were conducted. The experimental design of experiment A was a randomized complete block of a split-split plot, with ozone (three levels: 0.00, 0.06, and 0.12 μl L^{-1}) as the main factor, cultivar (two levels: Alaska and Bounty) as the subplot factor, and powdery mildew inoculation (two levels: inoculated or noninoculated) as the sub-subplot factor. When the effect of ozone on leaf colonization by the powdery mildew fungus was analyzed, noninoculated plants were deleted from the data set. Ten plants were used per treatment, for a total of 60 plants per cultivar. Plant positions were not changed after the plants were assigned to chambers at the beginning of exposure. Plants were inoculated at the end of the fifth day of fumigation.

The experimental outline of experiment B was also a randomized complete block but of a complete 3^2 factorial split plot, with preinoculation and/or postinoculation fumigations as main factors (each at three ozone levels: 0.00, 0.06, and 0.12 μl L^{-1}) and cultivar (two levels: Alaska and Bounty) as subplot factor. Plants were exposed to ozone for 5 days before, after, or both before and after inoculation with the pathogen. To achieve this arrangement, appropriate plants were moved to chambers with different ozone levels after plants were inoculated. In this experiment, all plants were inoculated with *E. p. pisi* conidia. Seven plants were used per combination, for a total of 63 plants per cultivar. The pots within each chamber were moved daily during the exposure period to rerandomize plant position.

Both experiments were conducted twice. Different chambers were used for a given ozone treatment across repeats in each experiment.

Statistical analysis. The general linear models procedure of SAS (SAS Institute Inc., Cary, NC) was used for analyses of variance on all experimental data except the pisatin assay data. In addition, the nontransformed data were regressed on time after planting to calculate rates of stem growth. Foliar ozone injury data and fungal colonization data were transformed by the arcsine-square root transformation and regressed on time after start of fumigation or time after inoculation, respectively. Slopes of the regression lines obtained for each experimental plant were subjected to analysis of variance to examine possible differences in rates of development of ozone-induced foliar injury or fungal colonization.

RESULTS

Shoot growth. Ozone fumigation significantly reduced stem height ($P = 0.040$) and stem growth rate ($P = 0.002$) of Alaska seedlings, whereas infection by *E. p. pisi* significantly increased these parameters ($P = 0.033$ and 0.011, respectively) (Table 1). There were no significant interactions between the two stresses on Alaska plants for these growth variables. Stem heights of Bounty plants were not affected by ozone or infection in experiment A (Table 1), but preinoculation fumigation in experiment B significantly lowered stem growth rate ($P = 0.042$) (Table 2).

Exposure to ozone before inoculation with the fungus significantly reduced shoot dry weights of both cultivars ($P = 0.024$ for Alaska and 0.042 for Bounty) (Table 2). Bounty plants exhibited an additional decrease in dry weight associated with infection by the pathogen, but this effect was not statistically significant ($P = 0.116$) (Table 1).

Neither exposure to ozone nor infection with *E. p. pisi* had a significant effect on total leaf area of either cultivar (Table 1).

Foliar ozone injury. The foliage of Alaska seedlings exhibited significantly more ozone injury than the foliage of Bounty plants (Table 1). Leaves inoculated with *E. p. pisi* showed significantly less foliar ozone injury than noninoculated leaves for cultivar Alaska ($P = 0.002$) (Table 1). Plants exposed to ozone after inoculation with the pathogen in experiment B had less foliar ozone injury than those exposed before inoculation. This result was also reflected in the rates of development of ozone injury: Timing of inoculation had a significant effect on injury rate for both cultivars ($P = 0.003$ and 0.023 for Alaska and Bounty, respectively) (Table 2).

Colonization of leaves by the fungus. Bounty was significantly more resistant to powdery mildew than Alaska (Table 1). Fumigation with ozone at 0.12 $\mu l\ L^{-1}$ suppressed leaf colonization in Bounty ($P = 0.043$) but not in Alaska (Table 1). In experiment B, the suppression of leaf colonization associated with ozone exposure was marginally significant for both Alaska ($P = 0.058$) and Bounty ($P = 0.087$) in the preinoculation fumigation treatment but not the postinoculation treatment (Table 2). Also, the rate of leaf colonization by the pathogen was slowed more by the preinoculation fumigation than by the postinoculation fumigation (Table 2). This difference between fumigation treatments was marginally significant for Alaska ($P = 0.056$) and highly significant for Bounty ($P = 0.010$).

Pisatin assay. Pisatin was not detected in leaf tissue of noninoculated plants exposed to ozone at 0.06 or 0.12 $\mu l\ L^{-1}$ (Table 3). In plants colonized by *E. p. pisi,* however, the phytoalexin accumulated to a detectable level in all leaf samples. However, pisatin concentration varied considerably among the four samples tested for each treatment combination, as indicated by the standard deviations (Table 3). Pisatin concentrations tended to be lower in colonized leaves of Alaska peas than in those of Bounty peas. Infected leaves of Bounty peas not exposed to ozone (control) accumulated about the same amounts of pisatin as those exposed at the 0.06 $\mu l\ L^{-1}$ level. The results suggest that exposure to ozone at 0.12 $\mu l\ L^{-1}$ strongly suppressed pisatin levels of infected Bounty plants (Table 3).

DISCUSSION

The suppression of stem growth and shoot dry weight accumulation observed for Alaska plants exposed to ozone was expected from results of other studies (20,21). Infection of plants with *E. p. pisi* appeared to stimulate stem growth in this study, in contrast to a field study that found that infection with the powdery mildew pathogen reduced stem height of pea cultivars Alsweet and Perfected Freezer (6). The apparent attenuation of the ozone effect on stem height and shoot dry weight associated with the additional stress of the fungal infection may have been the result of restricted ozone uptake caused by a decrease in stomatal conductance of the infected tissue, as reported for wheat powdery mildew (17).

Cultivar Alaska was significantly more sensitive to ozone than

TABLE 1. Effect of ozone fumigation and powdery mildew infection on growth, ozone-induced injury, and fungal colonization of seedlings of two pea cultivars (experiment A)[a]

Ozone treatment[b]										
Preinoculation ($\mu l\ L^{-1}$)	Postinoculation ($\mu l\ L^{-1}$)	Mildew[c]	Stem height (mm)	Stem growth rate (mm/day)	Shoot dry weight (g)	Total leaf area (cm^2)	Ozone injury[d] (%)	Injury rate[e] (%/day)	Leaf colonization[f] (%)	Colonization rate[g] (%/day)
Cultivar Alaska[h]										
			*+	**++			**			
0.00	0.00	No	391	38.7	1.05	104.7	0	0.00	—	—
0.00	0.00	Yes	406	42.3	1.05	104.7	0	0.00	57	0.17
0.06	0.06	No	377	38.7	0.99	101.3	24	0.08	—	—
0.06	0.06	Yes	412	43.1	1.01	101.1	13	0.05	65	0.21
0.12	0.12	No	355	35.4	0.78	91.6	63	0.08	—	—
0.12	0.12	Yes	388	39.5	0.87	92.9	45	0.09	50	0.17
Cultivar Bounty[h]										
									*	
0.00	0.00	No	130	11.2	0.75	74.4	0	0.00	—	—
0.00	0.00	Yes	131	11.4	0.71	77.4	0	0.00	19	0.12
0.06	0.06	No	135	11.7	0.80	79.5	2	0.01	—	—
0.06	0.06	Yes	134	11.9	0.70	78.5	5	0.02	21	0.13
0.12	0.12	No	123	10.0	0.64	74.2	39	0.05	—	—
0.12	0.12	Yes	123	10.5	0.58	65.9	27	0.05	10	0.08

[a]Data are means of 10 plants. Measurements were taken at time of harvest (rates were derived from linear regressions).
[b]Ozone exposure levels for 5 days (6 h/day) before and after inoculation, respectively.
[c]Inoculation with conidia of *Erysiphe polygoni* f. sp. *pisi* after 5 days of ozone exposure.
[d]Percentage of leaf area injured by ozone (mean of oldest four leaves).
[e]Rate of foliar ozone injury development (mean leaf proportion injured per day).
[f]Percentage of leaf area colonized by *E. p. pisi* (mean of all inoculated leaves [oldest four leaves]).
[g]Rate of leaf colonization (mean leaf proportion colonized per day).
[h]Significance of ozone effect is indicated with * and ** ($P < 0.05$ and $P < 0.01$, respectively). Significance of mildew effect is indicated with + and ++ ($P < 0.05$ and $P < 0.01$, respectively). The ozone × mildew interaction was not significant for any variable. The cultivar effect was significant ($P < 0.01$) for all variables. The cultivar × mildew interaction was significant ($P < 0.01$) for stem height, stem growth rate, shoot dry weight, ozone injury, and injury rate. The cultivar × ozone interaction was significant ($P < 0.05$) for injury rate. The cultivar × ozone × mildew interaction was significant for ozone injury ($P < 0.05$) and injury rate ($P < 0.01$).

Bounty, which showed almost no foliar injury after 10 days of exposure to the pollutant at 0.06 $\mu l\ L^{-1}$. These results are consistent with an earlier study that demonstrated that pea cultivars differ significantly in sensitivity to ozone (26). The reason for these differences is not well understood, although the existence of a cellular "compensation system" against ozone damage has been proposed (4), which might be more extensive in certain cultivars than in others.

The significant suppression of foliar ozone injury due to infection of pea plants with *E. p. pisi* is in agreement with the results of another study (9) that showed that infection with the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici* protected wheat leaves from ozone injury. The authors suggested that the source of the protection might have been a "diffusible substance" liberated from the infecting fungus that directly or indirectly inhibited ozone damage. However, reduced pollutant uptake as a result of stomatal closure caused by powdery mildew infection, which has been reported for wheat (17), could also explain the decreased ozone injury (25).

Exposure of pea plants to ozone at a concentration of 0.12 $\mu l\ L^{-1}$ (but not 0.06 $\mu l\ L^{-1}$) inhibited leaf colonization by the powdery mildew fungus. When barley plants infected with barley powdery mildew were exposed to ozone, single colonies were larger but there were fewer infections overall (10). A similar decrease in the number of successful infections may account for the less extensive colonization of pea leaves observed in this study in seedlings exposed to ozone compared to those not exposed. If so, this would suggest that ozone affected the plant rather than the pathogen: Plants exposed to ozone before inoculation experienced substantially less colonization, whereas fumigation concurrent with incubation of the pathogen had a much smaller effect.

How ozone fumigation suppressed the colonization of pea leaves by *E. p. pisi* is unknown. Increased ozone injury may have had a direct effect on the growth of this biotrophic pathogen, in that leaves injured by ozone may be less suitable as a host for the fungus. However, as mentioned above, ozone levels that caused partial chlorosis of barley plants stimulated rather than inhibited the growth of single colonies of barley powdery mildew (10). This apparent lack of inhibition was explained by the colonization of epidermal cells rather than mesophyll cells, which are the main sites of visible ozone injury (10).

Thus, the increase in resistance observed in this study must have been caused by a change in the physiology of the host plant. A decrease in photosynthates in older leaves, caused by an ozone-induced decrease in photosynthesis (18), may have slowed the colonization of leaves by the pathogen. Or exposure to ozone at 0.12 $\mu l\ L^{-1}$ for 5 days may have induced production of one or more cellular compounds in the stressed plants that may have lowered the ability of *E. p. pisi* to colonize the host tissue.

The pea phytoalexin pisatin is one such plant stress compound. Our assays detected pisatin accumulation in plants infected with the pathogen, as has been reported previously (24). Pisatin did not accumulate in the leaves of plants exposed to ozone only, however, which indicates that this phytoalexin does not play a role in the resistance phenomenon observed in this study. The concentration of pisatin may have fallen below the detection limit (about 1 μg per gram of dry weight) during the experiment after a hypothetical increase during the first few days of fumigation. However, pisatin concentrations increased linearly for several days

TABLE 2. Effect of ozone fumigation before and/or after inoculation with *Erysiphe polygoni* f. sp. *pisi* on growth, ozone-induced injury, and fungal colonization of seedlings of two pea cultivars (experiment B)[a]

Ozone treatment[b] ($\mu l\ L^{-1}$)		Stem height (mm)	Stem growth rate (mm/day)	Shoot dry weight (g)	Total leaf area (cm^2)	Ozone injury[c] (%)	Injury rate[d] (%/day)	Leaf colonization[e] (%)	Colonization rate[f] (%/day)
Preinoculation	Postinoculation								
Cultivar Alaska[g]									
				*		*	**		
0.00	0.00	511	42.3	1.37	113.6	0	0.00	65	0.18
0.00	0.06	501	41.5	1.36	113.1	0	0.00	61	0.18
0.00	0.12	493	40.3	1.42	113.2	12	0.05	52	0.15
0.06	0.00	493	40.8	1.31	111.1	1	0.01	58	0.17
0.06	0.06	502	41.0	1.39	113.1	3	0.03	61	0.18
0.06	0.12	497	40.8	1.37	111.2	14	0.05	57	0.16
0.12	0.00	474	38.7	1.18	102.5	27	0.05	52	0.14
0.12	0.06	483	39.3	1.21	104.0	33	0.05	47	0.12
0.12	0.12	468	38.4	1.24	104.4	35	0.06	41	0.11
Cultivar Bounty[g]									
			*	*		*	*		**
0.00	0.00	148	10.5	0.75	76.2	0	0.00	28	0.11
0.00	0.06	147	10.6	0.79	74.9	0	0.00	25	0.11
0.00	0.12	147	10.7	0.75	70.4	2	0.01	21	0.10
0.06	0.00	144	10.3	0.77	77.4	0	0.00	27	0.12
0.06	0.06	144	10.3	0.78	74.0	0	0.00	24	0.11
0.06	0.12	148	10.5	0.77	70.4	3	0.01	21	0.10
0.12	0.00	142	9.9	0.76	72.2	8	0.03	10	0.07
0.12	0.06	144	10.3	0.73	73.1	10	0.03	13	0.08
0.12	0.12	142	9.8	0.71	64.9	11	0.04	6	0.06

[a] Data are means of seven plants. Measurements were taken at time of harvest (rates were derived from linear regressions). All plants were inoculated with conidia of *E. p. pisi* after 5 days of ozone exposure.

[b] Ozone exposure levels for 5 days (6 h/day) before and after inoculation, respectively.

[c] Percentage leaf area injured by ozone (mean of oldest four leaves).

[d] Rate of foliar ozone injury development (mean leaf proportion injured per day).

[e] Percentage of leaf area colonized by *E. p. pisi* (mean of all inoculated leaves [oldest four leaves]).

[f] Rate of leaf colonization (mean leaf proportion colonized per day).

[g] Significance of effect of preinoculation ozone is indicated with * and ** ($P < 0.05$ and $P < 0.01$, respectively). The interaction of preinoculation and postinoculation ozone was significant ($P < 0.01$) for injury rate for cultivar Alaska. The cultivar effect was significant ($P < 0.01$) for all variables. The preinoculation ozone × cultivar interaction was significant ($P < 0.01$) for stem height, stem growth rate, shoot dry weight, ozone injury, and leaf colonization. The postinoculation ozone × cultivar interaction was significant ($P < 0.01$) for total leaf area, ozone injury, and leaf colonization. The preinoculation ozone × postinoculation ozone × cultivar interaction was not significant for any variable.

TABLE 3. Pisatin concentration in the foliage of pea seedlings exposed to ozone and/or infected with powdery mildew

Ozone treatment[a] Preinoculation (μl L^{-1})	Postinoculation	Mildew[b]	Pisatin concentration[c] (μg g^{-1})
Cultivar Alaska			
0.00	0.00	No	Not detectable
0.00	0.00	Yes	28.5 ± 18.1
0.06	0.06	No	Not detectable
0.06	0.06	Yes	13.0 ± 9.1
0.12	0.12	No	Not detectable
0.12	0.12	Yes	21.8 ± 15.5
Cultivar Bounty			
0.00	0.00	No	Not detectable
0.00	0.00	Yes	64.5 ± 17.4
0.06	0.06	No	Not detectable
0.06	0.06	Yes	63.5 ± 29.5
0.12	0.12	No	Not detectable
0.12	0.12	Yes	22.2 ± 35.2

[a] Ozone exposure levels for 5 days (6 h/day) before and after inoculation, respectively.
[b] Inoculation with spores of *Erysiphe polygoni* f. sp. after 5 days of ozone exposure.
[c] Pisatin concentration ± standard deviation of the mean in total sample (mean of four replicates).

after the application of $CuCl_2$ to pea pods (2) and pea seeds (32), which suggests that pisatin synthesis induced by several days of ozone exposure would be detectable for some time, especially when the ozone stress was renewed daily over the whole course of the experiment.

Ozone has been reported to induce synthesis of phytoalexins in other legume species (12,29,31). However, plants in those experiments were exposed to much higher concentrations of ozone (0.25–0.80 μl L^{-1} for a few hours) than those used in this research. Thus, an acute ozone stress may be necessary to induce synthesis of phytoalexins. Furthermore, pea plants may respond to ozone stress differently than other legumes, or a different phytoalexin that was not detected by our assay may have been induced in the pea plants.

Pisatin levels in leaf tissue samples indicated that the extent of leaf colonization by the fungus, not the ozone stress, determined the concentration of the phytoalexin. This result supports the conclusion that pisatin was not involved in the observed increase in resistance.

This study shows that ozone and powdery mildew are interactive plant stresses that have a less-than-additive effect on pea plants. Leaves infected with *E. p. pisi* had significantly less foliar ozone injury than noninoculated leaves. Exposure to ozone at 0.12 μl L^{-1} (but not 0.06 μl L^{-1}) significantly suppressed colonization of leaves by the pathogen, perhaps by decreasing infection efficiency. This observation could have important implications for slowing the spread of the pathogen by increasing the rate-reducing resistance (28,30).

Ozone concentrations in ambient air of most agricultural areas in the United States (0.04–0.06 μl L^{-1} [8]) are probably not high enough to affect powdery mildew of pea plants. An increase in ambient ozone concentrations might raise the resistance of some pea cultivars to *E. p. pisi* infection by a mechanism other than increased pisatin levels in the affected plants.

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