# Effects of Hydrogen Peroxide Seed-Disinfestation Treatments on Germination and Development of *Pisum sativum*

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**ABSTRACT**

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Two groups of seeds of *Pisum sativum* 'Alaska' and 'Garfield' were treated with 5, 10, 15, 20, 25, or 30% hydrogen peroxide for 7.5, 15, 30, 45, or 60 min. One group was placed in germination plates to determine percentage of germination, radicle length, size of root-hair mass, and percentage of contamination after 5 days (Alaska) or 6 days (Garfield); the other group was planted in a greenhouse to determine stem length, number of nodes, number of reproductive nodes, root volume, root weight, or top weight at maturity (after 55 days for Alaska or 72 days for Garfield). Although most treatments enhanced plant performance for both cultivars, there were substantial differences for the various types of growth measured. Considering all measurement categories collectively, the single treatment that was as good or better than other treatments for both cultivars was immersion of seeds in 30% hydrogen peroxide for 45 min.

Seeds of higher plants carry bacterial and fungal contaminants, which sometimes are symbionts or pathogens, on and within their surfaces (4). Most seeds also have surface irregularities that make disinfestation difficult. Numerous biocidal substances have been used for seed disinfestation, but most of them either fail to penetrate surface irregularities satisfactorily or leave chemical residues that can interfere with studies of the effects of other toxicants. When legume plants are used as the test system for evaluating effects of toxicants on natural nitrogen fixation, it is especially desirable to eliminate extraneous nodulating organisms before the seeds are planted. The common disinfestants ethyl alcohol, sodium hypochlorite, and mercuric chloride accomplish this when used properly, but the problem of chemical residue remains.

Although seldom used, hydrogen peroxide ($H_2O_2$) has long been known to be effective for seed disinfestation (7,11,12). Residues decompose rapidly to water and oxygen and usually without apparent damage to treated seeds (10). However, elevated temperatures have led to legume injury by 10–15% $H_2O_2$ for 30 min (12), and treatment duration has been more critical than concentration of $H_2O_2$ in producing injury (P. S. Dhillon, *unpublished*).

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Beneficial effects other than seed disinfestation have been observed with $H_2O_2$; eg, weak solutions (1% or less) have increased germination rates for seeds of *Abies* (2), *Acer* (11), *Cryptomeria* (5), *Larix* (10), *Pinus* (2,5), and *Pseudotsuga* (2,8). However, harsh treatments such as 30% $H_2O_2$ for 30 min (7) and 35% for 60 min (11) have also increased germination of seeds of various trees and shrubs. Germination and emergence were improved for navy bean (*Phaseolus vulgaris* 'Seafarer'), soybean (*Glycine max* 'Corsoy'), and sugar beet

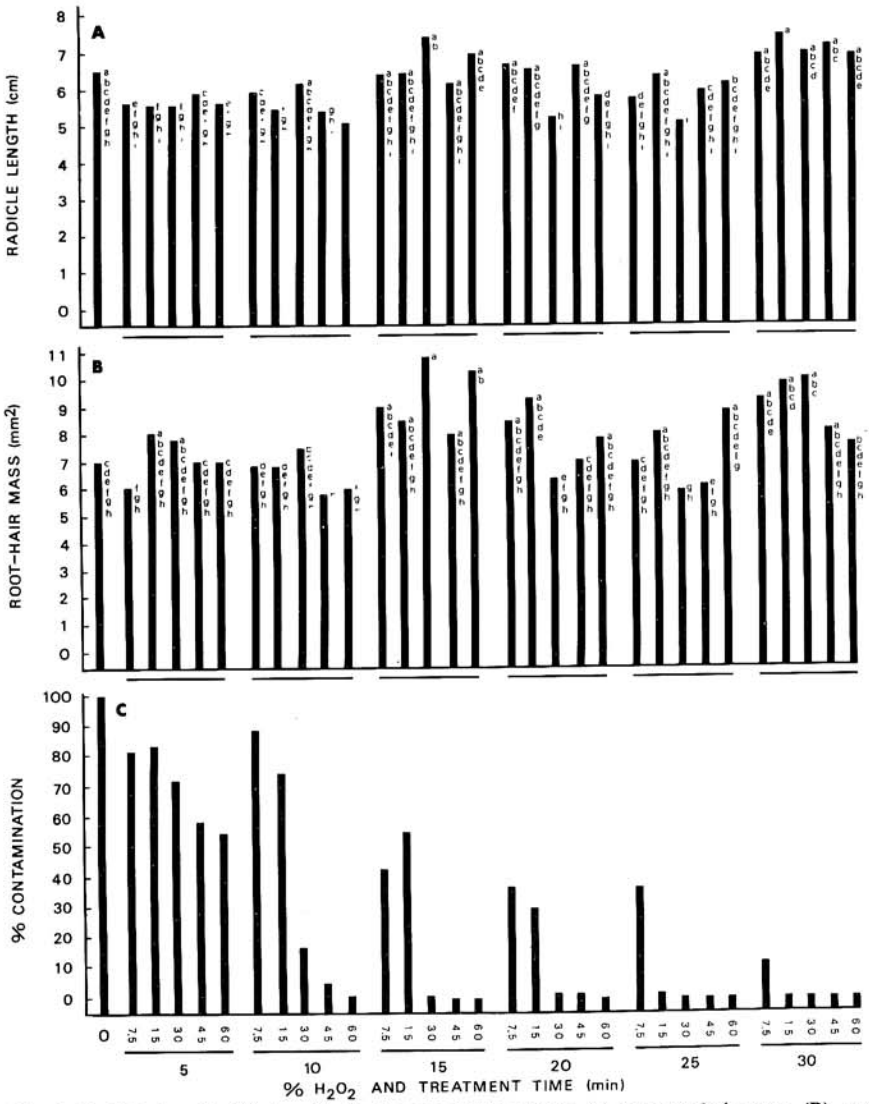


**Fig. 1.** Radicle length **(A)**, longitudinal cross-sectional area of the root-hair mass **(B)**, and percentage of seeds contaminated with bacteria or fungi **(C)** 5 days after Alaska pea seeds were soaked in 5, 10, 15, 20, 25, or 30% hydrogen peroxide ($H_2O_2$) for 0, 7.5, 15, 30, 45, or 60 min. Each column is an average for 150 seeds. Within each graph, columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

(*Beta vulgaris* US H20) when seeds were soaked in as little as 0.25% $H_2O_2$ for 4 hr—unless the seeds were dried before planting, in which case cracking and reduced germination occurred (9). Soaking *P. vulgaris* seeds in dilute $H_2O_2$ for up to 24 hr before, during, or after soaking in water prevented the usual water-soaking injury (reduced germination and growth of survivors) and was slightly stimulatory to subsequent growth (6).

Despite the variation in results reported for $H_2O_2$, it was selected as the most promising compound for development of a rapid and reliable method for disinfesting legume seeds without leaving a chemical residue. Experiments were conducted to identify optimum conditions for satisfactory disinfestation and for rapid development of plants of two cultivars of the field pea *Pisum sativum* L.

## MATERIALS AND METHODS

The spring pea cultivars Alaska and Garfield were selected for study. Seeds were not sized because earlier tests (*unpublished data*) had indicated that seed size exerted negligible effects. Sterile, double-distilled water (sdd$H_2O$) was used for all rinses. For the germination tests, untreated control seeds of Alaska and Garfield (stored 6 mo at room temperature) were soaked for 24 or 1 hr, respectively, in sdd$H_2O$ and placed in germination plates while still wet. For the greenhouse tests, untreated control seeds were not presoaked but planted dry. These manipulations were based on earlier best-performance tests (*unpublished data*).

Germination tests were conducted in sterile, polystyrene petri plates (15 mm deep × 100 mm square) on sterilized sheets of seed-germination paper cut to fit into the plate and supported on pleated, 8- to 9-mm ribbons of germination paper turned on edge. The ribbons of supporting paper were just wide enough (the vertical dimension when in the plate) to hold the seeds in firm contact with the plate lid without crushing them. In three separate tests, 50 seeds of each cultivar were immersed in 5, 10, 15, 20, 25, or 30% $H_2O_2$ (Mallinckrodt 30% analytical reagent) for 7.5, 15, 30, 45, or 60 min. Five seeds of one cultivar and one treatment were then arranged in a row along one side of the supported germination paper (2 cm from the edge of the plate) in each of 10 plates.

During incubation, the sides of the plates containing the seeds were elevated to an angle of 45° from the horizontal, which encouraged geotropic straightening of radicles and thereby facilitated length measurements. The lower side of each plate rested in sdd$H_2O$; all plates were incubated in darkness at 20 ± 1 C. Percentage of germination, radicle length, and cross-sectional area of root-hair development were recorded at intervals of 48, 72, 96, and 120 hr after completion of the soak period. (The "root-hair area" was a relative figure: ie, the product of maximum length and maximum width of the root-hair mass.)

Disinfestation effects were evaluated in sterile, round, polystyrene petri plates (15 × 100 mm) containing 20 ml of standard nutrient agar or potato-dextrose agar. To avoid unacceptable $H_2O_2$ injury, which occurs when seeds are disinfested near the end of the optimum water-soak period (*unpublished data*), all $H_2O_2$ treatments were applied to dry seeds, and the water-soak period was completed in sdd$H_2O$ after four rinses with sdd$H_2O$. Fifty seeds of each cultivar for each growth medium were treated in each of the six $H_2O_2$ concentrations for each of the five periods of time. Then five seeds were placed in a circle in each of 10 plates for each treatment and each growth medium as the agar was solidifying so that each seed was completely immersed in the agar. All plates were incubated in darkness at 25 ± 1 C. Bacterial and fungal contamination was recorded for each medium, each treatment, and every seed at intervals of 24, 48, 72, and 96 hr after treatment.

Greenhouse studies of effects on plant growth were conducted with seeds that had been subjected to the various $H_2O_2$ treatments while dry, then rinsed four times in sdd$H_2O$, drained on filter paper, and dried again in a laminar-flow gnotobiotic chamber. Seeds were planted in holes 3 cm deep in 15-cm plastic pots containing an autoclaved potting mix of 1:1 (v/v) silica sand (99.7% chemically pure) and horticultural grade vermiculite. Each seed was placed on 1 g of Nitragin (a

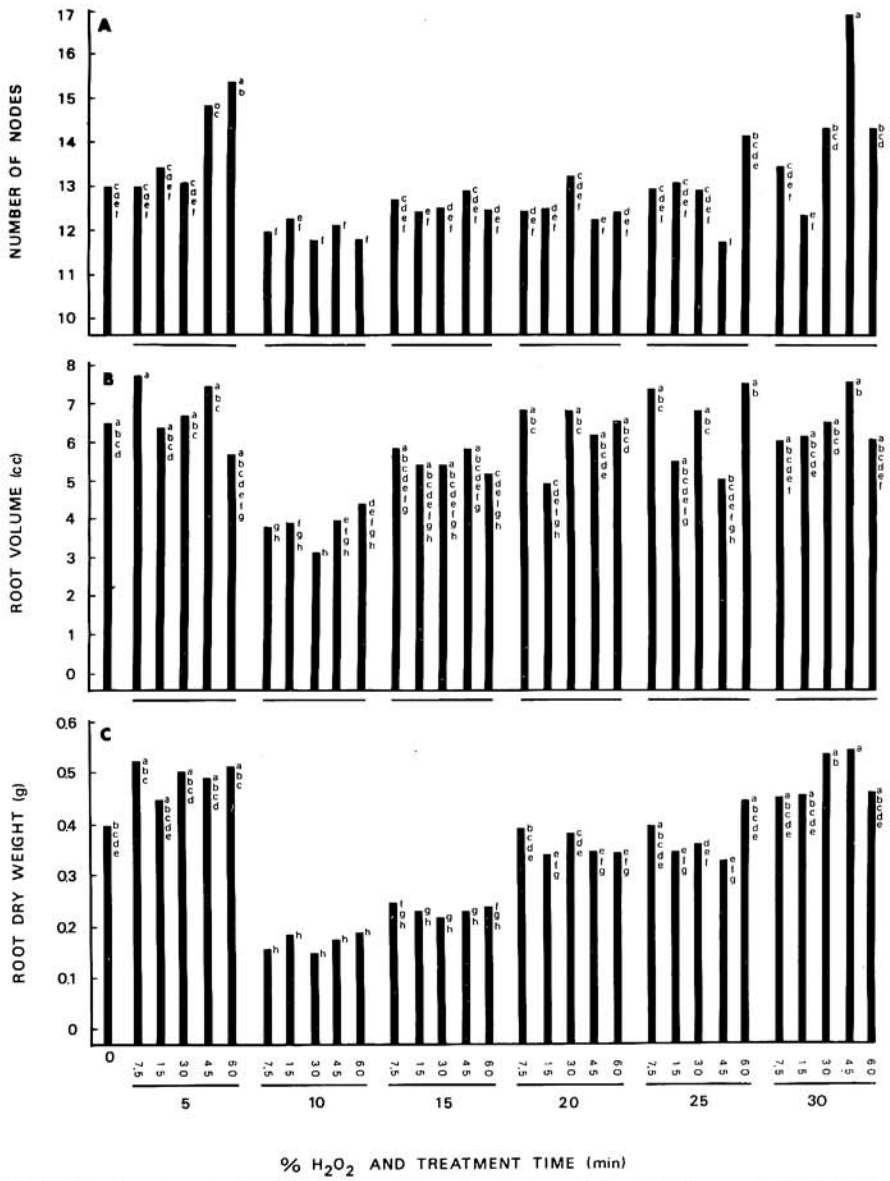


**Fig. 2.** Number of nodes **(A)**, root volume **(B)**, and root dry weight **(C)** 55 days after Alaska pea seeds were soaked in 5, 10, 15, 20, 25, or 30% hydrogen peroxide ($H_2O_2$) for 0, 7.5, 15, 30, 45, or 60 min. Each column is an average for 24 treated plants or 48 untreated control plants. Within each graph, columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

peat product of the Nitragin Co., Milwaukee, WI, containing four strains of *Rhizobium leguminosarum* Frank rated at $10^8$ bacteria per gram) when planted. Nutrition was supplied via watering as needed with N-free Dart and Pate (3) nutrient solution amended with 1 ppb of cobalt as $CoCl_2 \cdot 7H_2O$ (1).

Plants were grown under a 14-hr metal-halide photoperiod, a temperature regime of 25.6 C day and 14.4 C night, with corresponding relative humidities of 45 and 80%. Plants were harvested at maturity, and data were collected on plant height (stem length), total number of reproductive nodes, root volume, root dry weight, and top dry weight (including pods and seeds).

## RESULTS AND DISCUSSION

**Alaska.** Germination was high (84–100% after 5 days) for controls and all $H_2O_2$ treatments; differences were not significant, and data are not presented. No single treatment resulted in significantly greater radicle development than occurred with several other treatments (Fig. 1A), but numerically longest radicles were found where seeds had been treated with 30% for 15 min, then 15% for 30 min. Two $H_2O_2$ treatments resulted in significantly greater root-hair development than occurred with untreated controls (Fig. 1B). Greatest numerical values were recorded for 15% $H_2O_2$ for 30 and then 60 min.

All untreated seeds were contaminated with fungi or bacteria, but contamination decreased with increasing severity of the $H_2O_2$ treatment (Fig. 1C). Contamination was eliminated with 15% for 45 min or more, 20% for 60 min, 25% for 30 min or more, and 30% for 15 min or more. Considered collectively, and based on significant differences and numerical values, these data show that best performance of Alaska seeds (successful disinfestation, longest radicle, and most extensive development of root hairs) occurred with an $H_2O_2$ treatment of 15% for 30 min. Stem length varied between 89.5 and 103.7 cm after 55 days of growth in the greenhouse. None of the $H_2O_2$ treatments affected stem length (*data not presented*). Increased numbers of nodes were associated with prolonged treatment with 5 and 30% $H_2O_2$ (Fig. 2A). The decrease in nodes with the 10% treatment is not understood. Increased numbers of reproductive nodes were associated with the longer 5 and 30% treatments (*data not presented*). Root volume varied widely, but 10% $H_2O_2$ significantly and consistently decreased root volume (Fig. 2B). These differences were more prominent in root dry-weight figures (Fig. 2C), where 5 and 30% treatments produced root development that was as good or greater than occurred with untreated seeds.

Greatest reductions in root weights were associated with the 10 and 15% treatments, with performance improving slightly as the severity of the $H_2O_2$ treatment was increased. Reductions in root dry weight by 10 and 15% $H_2O_2$ but not by higher concentrations cannot be explained with available data. Top-weight figures (*data not presented*) paralleled root weights. Considered collectively, and based on significant differences and numerical values, the data indicate that although unknown factors obviously are active, best performance of Alaska pea plants occurred with a preplant seed treatment of 5 or 30% $H_2O_2$.

The best treatment for enhancement of germination and early seedling development of Alaska peas was immersion of seeds in a 15% solution of $H_2O_2$ for 30 min, whereas the best treatment for prolonged benefits in plant growth appeared to be preplant immersion in 30% $H_2O_2$ for 45 min.

**Garfield.** Germination of seeds ranged from 67–100% after 6 days, with no apparent correlation with $H_2O_2$ treatments (*data not presented*). Several treatments resulted in significantly greater radicle lengths than produced on untreated seeds (Fig. 3A). Greatest numerical value was obtained with 25% treatment for 30 min. Seeds treated with 30% $H_2O_2$ for 15 min produced significantly greater masses of root hairs than control seeds (Fig. 3B). Eighty-six percent of untreated seeds were contaminated with fungi or bacteria, but contamination decreased with increased severity of $H_2O_2$ treatments (Fig. 3C). Contaminating organisms were eliminated with 20 and 25% $H_2O_2$ for 30 min or more and with 30% for 45 min or more exposure. Considered collectively, and based on significant differences and numerical values, these data indicate that the best performance of Garfield pea

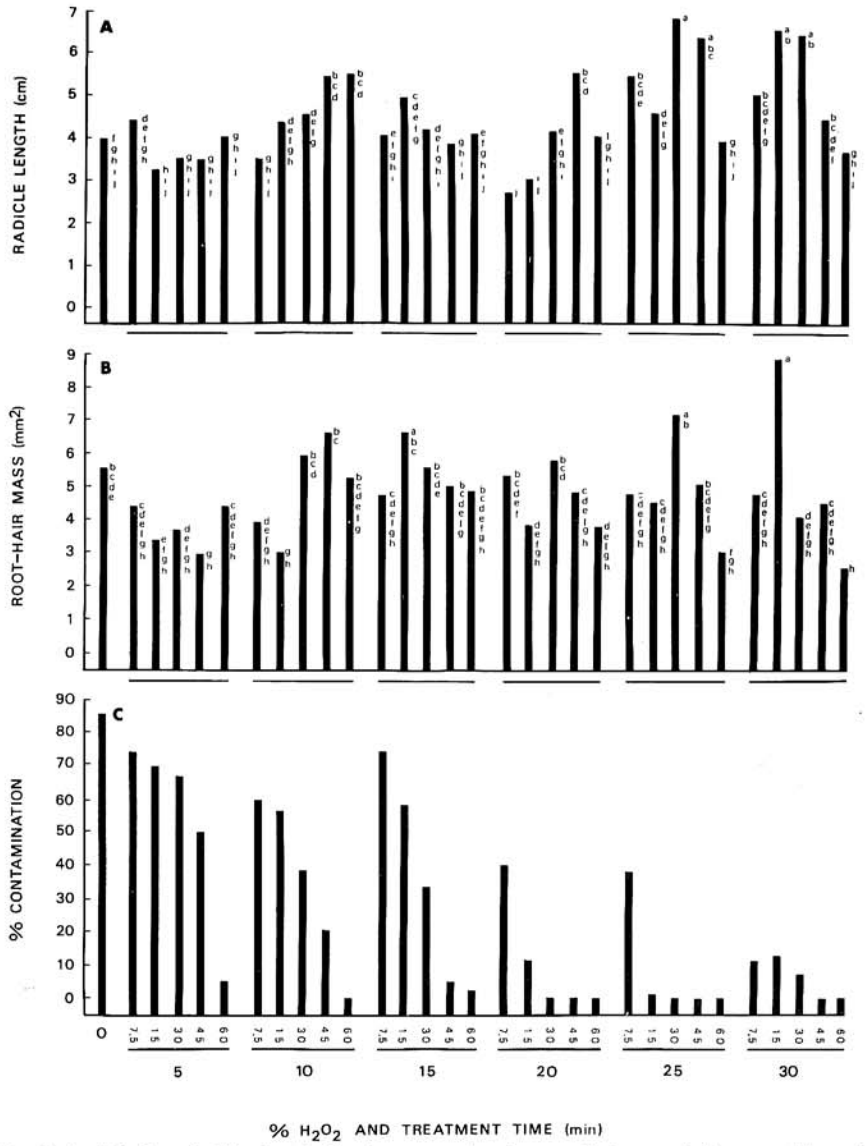


**Fig. 3.** Radicle length **(A)**, longitudinal cross-sectional area of the root-hair mass **(B)**, and percentage of seeds contaminated with bacteria or fungi **(C)** 6 days after Garfield pea seeds were soaked in 5, 10, 15, 20, 25, or 30% hydrogen peroxide ($H_2O_2$) for 0, 7.5, 15, 30, 45, or 60 min. Each column is an average for 150 seeds. Within each graph, columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

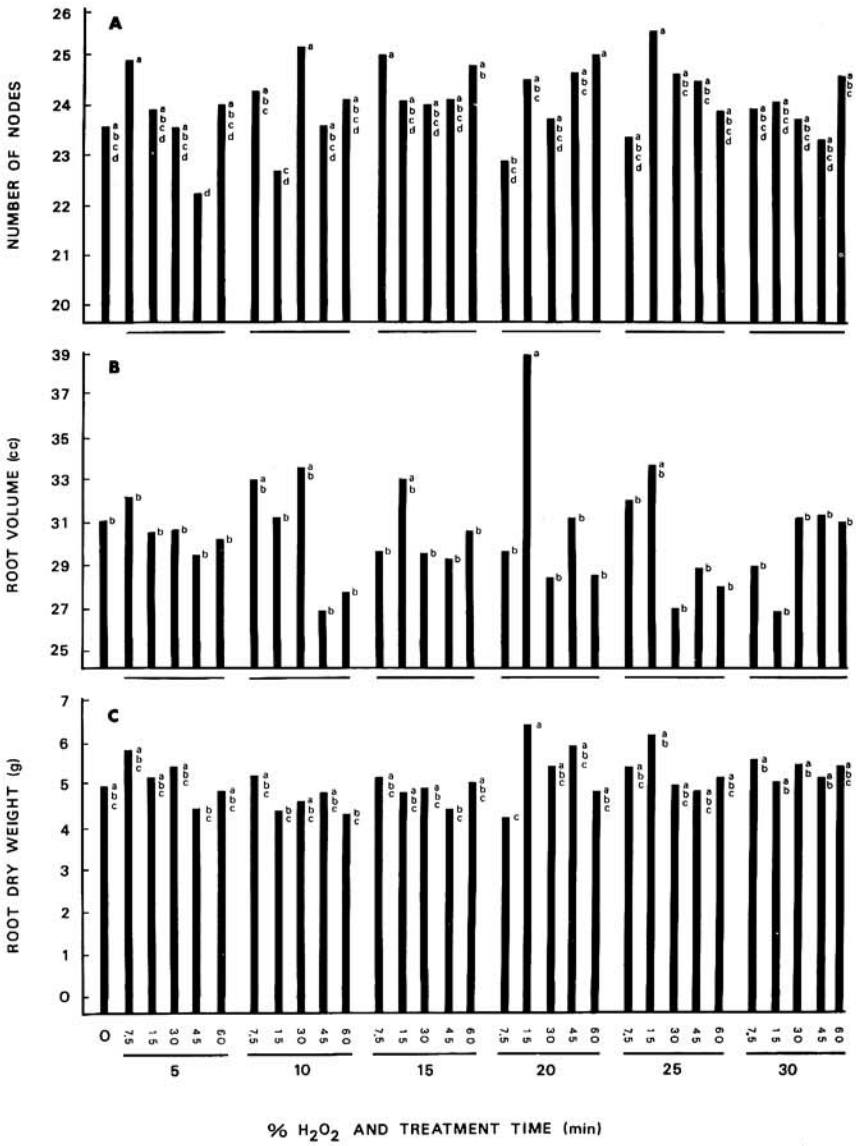


**Fig. 4.** Number of nodes **(A)**, root volume **(B)**, and root dry weight **(C)** 72 days after Garfield pea seeds were soaked in 5, 10, 15, 20, 25, or 30% hydrogen peroxide ($H_2O_2$) for 0, 7.5, 15, 30, 45, or 60 min. Each column is an average for 24 treated plants or 48 untreated control plants. Within each graph, columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

seeds occurred with an $H_2O_2$ treatment of 25% for 30 min.

Stem length varied between 216.0 and 258.6 cm after 72 days of growth in the greenhouse. All treatments with $H_2O_2$ except 10 and 25% for 7.5 min resulted in numerically greater growth than that of untreated controls, and growth associated with treatments of 10% $H_2O_2$ for 60 min, 20% for 60 min, and 30% for 7.5 and 15 min was significantly greater than that of untreated controls (*data not presented*). $H_2O_2$ treatments frequently were associated with numerically greater numbers of nodes than developed on untreated controls, but these differences were not statistically significant (Fig. 4A). The same was true for numbers of reproductive nodes, even though the numerical scattering was greater (*data not presented*).

Root volume also varied widely (Fig. 4B). Only the 20% $H_2O_2$ treatment for 15 min resulted in significantly greater root volume than that of untreated controls; no treatments reduced root volume significantly. Root dry weights were not affected by the $H_2O_2$ treatments (Fig. 4C), but 20% for 15 min was numerically superior to controls and to all other treatments. Top weights varied more than root weights and did not correlate with $H_2O_2$ treatments (*data not presented*). No treatment produced top growth that was significantly different from that of controls, but 10% $H_2O_2$ for 30 min was greater numerically, followed by 20% for 15 min. Considered collectively, and based on significant differences and numerical values, these data suggest that despite wide variation in most categories of measurement, best overall performance of Garfield pea plants occurred with a preplant seed treatment of 20% $H_2O_2$ for 15 min or 10% for 30 min.

Considering the differences in all measurement categories, the best single treatment for enhancement of germination and early seedling development of Garfield peas was immersion of seeds in a 25% solution of $H_2O_2$ for 30 min; the best treatment for prolonged benefits in general plant growth was preplant immersion of seeds in 20% $H_2O_2$ for 15 min.

These results highlight the extensive variation in germination and growth of field peas, particularly when seeds are subjected to anaerobic preplant soaking in water and to disinfestation with $H_2O_2$. Best treatments for producing a large root-hair mass were not necessarily the best treatments for enhancing development of the total root system. Some of this variation no doubt resulted from the stimulatory effects of $H_2O_2$ at low and high concentrations and inhibitory effects at intermediate concentrations. In any case, the data show that field pea seeds can be disinfested without damaging them or leaving an undesirable residue.

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