Inquiry: The University of Arkansas Undergraduate Research Journal

Volume 6 Article 11

Fall 2005

Use of Calcium Hypochlorite as a Sanitizer for Seeds Used for Sprouting: Task #2; Impact: Improved Alfalfa Decontamination Technologies

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Damron, Emily; Klein, Carrie; Leach, Melissa; Mourot, Jordan; Murphy, Tom; Seamans, Amy; and Wilson, Ryan (2005) "Use of Calcium Hypochlorite as a Sanitizer for Seeds Used for Sprouting: Task #2; Impact: Improved Alfalfa Decontamination Technologies," *Inquiry: The University of Arkansas Undergraduate Research Journal*: Vol. 6 , Article 11.
Available at: http://scholarworks.uark.edu/inquiry/vol6/iss1/11

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This article is available in Inquiry: The University of Arkansas Undergraduate Research Journal: http://scholarworks.uark.edu/inquiry/vol6/iss1/11

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USE OF CALCIUM HYPOCHLORITE AS A SANITIZER FOR SEEDS USED FOR SPROUTING: TASK # 2

IMPACT: IMPROVED ALFALFA DECONTAMINATION TECHNOLOGIES

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Abstract:

Consumption of raw or lightly cooked alfalfa sprouts has been a concern of the U.S. Food and Drug Administration (FDA) in recent years due to connections between sprouts and food-borne illnesses. Researchers have identified contaminated seeds as the primary source of alfalfa sprouts contamination. Contamination of alfalfa seeds can originate in the field, harvesting, storing, or sprouting. Two pathogens of particular concern on alfalfa seeds are Escherichia coli O157:H7 (E. coli) and Salmonella. These pathogens are capable of producing biofilms that provide protection for individual cells and allow for survival in otherwise hostile environments, including some disinfectant washes. Other factors that contribute to contamination are the crevices of the seed surface, which provide opportunities for the protection of organisms. Various disinfection options have been evaluated and the use of a 20,000 ppm calcium hypochlorite ($Ca(OCl)_2$) solution is the most effective disinfectant for satisfying the requirements of Task 2.

Continued outbreaks of food poisoning indicate current disinfecting procedures are inadequate. In an effort to improve disinfection procedures, three bench scale apparatuses were constructed and tested to provide options for the commercial range of seed sanitation rates (from about 75 to 600 lb/week). Experiments were conducted to determine the disinfecting effectiveness of the apparatuses, as well as the current sanitization practices in industry. Experiments included dye removal tests where non-uniform dye removal indicated ineffective contacting. Similar experiments were performed using E. coli inoculating and post-contacting culturing.

One bench scale apparatus constructed was a model of the rotary drums widely used in industry. After a 30 minute treatment in the rotary drum, the seeds were found to be free of dye, indicating good contacting. The seeds were also sprouted showing sanitation did not damage the seeds. However, due to the capital expense of $14,000, the drum is not a viable option for small-scale sprout producers. For sprout growers who currently own rotary drums, drum use is recommended for seed sanitation.

Another bench scale apparatus was designed to improve the current industry practice of hand dunking seed-filled mesh draw-string bags. Through experimentation, it was determined the current hand dunking procedure, with little or no agitation, produces inadequate, non-uniform contact of the seeds and sanitizer. Therefore, the hand dunking procedure was modified to include agitation to effectively suspend the seeds throughout the bag to obtain good contact of the sanitizer solution with the seeds. The agitation-in-bag method is recommended for use in small volume sprout facilities, which currently employ the hand-dunking procedure for sanitizing. A modest investment of $113 is needed to implement the agitation-in-bag sanitizing method. Since no additional operating costs are accrued in implementation, no incremental costs are required.

Finally, an auger system was designed to sanitize one ton per hour of alfalfa seeds. The one ton per hour rate exceeds the demand of any individual sprout producer. Thus, the auger system is applicable to a partnership of sprout growers. Individual sprout growers within the mung bean industry, with much larger production volumes than the alfalfa industry, could economically use the auger system. However, the auger system can be scaled to sanitize any feed rate. A 1 3/8" diameter, 4' long auger bench scale model was constructed and tested at 4.5 lbs/hr rate with a contact time of 15 minutes. Scale-up of this bench scale sanitizer to a 2000 lb/hr rate requires a 16" diameter by 20' auger. The entire full-scale sanitizing system, which includes a vibrating screen washer, will handle 5,000,000 lb/yr of seeds, operating 8 hr/day, with a capital investment of $227,000 and an incremental operating cost (primarily labor) of $214,000/yr.

Introduction:

Alfalfa seeds are produced primarily in the U.S., Australia, and Canada[1]. In the U.S. alfalfa seed is primarily grown in the northwestern states of California, Idaho, Nevada, Oregon, Washington, and Wyoming[1]. Only a small portion of the seed produced is sprouted; the primary use of seed is to grow forage for the livestock industry. California is a major producer of sprouts in the U.S., accounting for $6.9 million of revenue to producers in 2000[2]. Current federal regulations for the sprout industry were written in a joint effort by the FDA and the California Department of Health Services (CDHS); therefore, the proposed design is targeted for implementation into a facility in California. Similarly, while sprouts are produced elsewhere in the world, the research presented is based on U.S. sprout growers only.

Most sprout associated illness outbreaks occur because of contaminated seed[11]. Contamination of seeds can occur during growth in the field, harvesting, storing, and sprouting. Sources of contamination have been found to be present in un-sanitized harvest equipment, conditioning equipment, and seed storage areas of the sprouting facility. Additional sources of contamination can also be the product of untreated irrigation water, animal waste, insects and other pests, and worker hygiene[1]. Localized contamination can easily be spread throughout the seed lot. Any damage to the seed coat by processing equipment could make the removal of microorganisms during subsequent steps more difficult.

Overview of Sprouting Industry

The sprouting industry consists of approximately 300 U.S. sprout growers. The largest scale producers include about 50 companies, which sanitize between 300 to 600 lbs of alfalfa seed per week, producing 3000 to 6000 lbs of sprouts per week[41]. The average sprout grower sanitizes about 75 lbs of alfalfa seed per week, producing 750 lbs of sprouts per week[41]. Given the range of demographics among sprout growers, three separate apparatuses were constructed in order to address the demands of the full range of sprout growers.

Alfalfa Seed Morphology

The morphology of alfalfa seed was studied to determine characteristics that might hinder the sanitization process. Alfalfa seed is yellowish-brown, kidney-shaped, and 1/12" long by 1/24" wide[3]. The alfalfa seed contains a lens, a small bump on the seed coat, which is the weakest point of the palisade layer of the seed and provides an easy entry point for water into the seed during germination[4]. Also, the seed coat of alfalfa seed is rough and creviced in comparison with other sprouted seeds, increasing the potential for contamination[5]. These surface crevices and seed coat imperfections provide sites where pathogens are concealed from the sanitizer solution.

Foodborne pathogens

Alfalfa seeds are commonly contaminated with *E. coli* and *Salmonella*, thus understanding the characteristics of these bacteria is important to sanitizing the seeds. *E. coli* and *Salmonella* are similar organisms. Both are prokaryotic, rod shaped, gram-negative bacteria with 90% of the same DNA[6]. The bacteria can double in number in as little as half an hour[7]. Optimal growth occurs between 25-40¾C and neutral pH which are the conditions used for sanitization. But the bacteria can grow in extremely harsh conditions, including temperatures as low as 4¾C and pH as high as 9.0. *Salmonella* is harder to kill than pathogenic strains of *E. coli* and requires a stronger or longer dose of the sanitizer to achieve the same kill level as *E. coli*. The bacteria have the ability to attach to the surface of a host. The surfaces of both alfalfa seeds and bacteria have a negative charge, which repel the attempts of the bacteria to attach to the alfalfa seeds. In order to overcome the negative charge, bacteria use a hydrophobic quality to attach to the hydrophobic seeds and pili to cover the surface of the bacteria[8]. Also, colonies of bacteria form biofilms on the seeds or equipment, which are highly resistant to sanitizers, protect the cells inside and provide nutrients. Given these obstacles, the alfalfa seed is difficult to effectively sanitize.

Current Sanitizing and Sprouting Facilities

Before improving the current sanitizing practices in industry, the procedures of several sprout growers were investigated to determine whether sources of error or contamination existed.

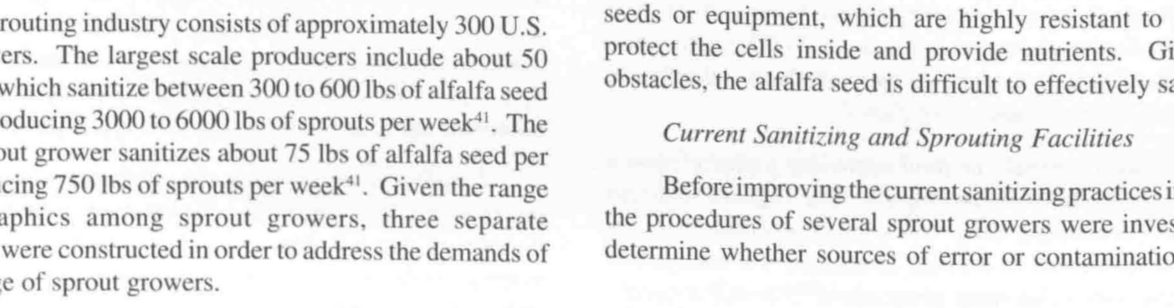


Figure 1. External, internal cross-section [3] and 40x view of alfalfa seed

Before the seeds are sanitized, the seeds are washed to wet the seed coats and to remove any dust[9]. In one sanitization cycle, small volume companies disinfect approximately 12 lbs of seed. The seeds are placed in a mesh bag and soaked in either a stainless steel or plastic tank (varying in size from 20 to 50 gallon) for 15 minutes in a 20,000 ppm $Ca(OCl)_2$ solution. The seeds are agitated periodically throughout the soaking process by using a paddle or by manually lifting the bag and replacing it in the solution. After sanitation, the seeds are rinsed until the chemical residue disappears and the chemical odor is gone. Some companies will then soak the seeds for two hours to assist germination, while others proceed directly into germination. Forty-eight hours into the sprouting process, companies collect spent irrigation water samples to be tested for the presence of pathogens. With the basis of the current procedures, improvements can be made to avoid contamination of the alfalfa seeds.

Outbreaks resulting from Alfalfa Sprouts

There has been increasing concern in the past decade regarding the consumption of raw sprouts due to the occurrence of outbreaks in the U.S. Approximately twenty *Salmonella* outbreaks, involving two deaths, and two *E. coli* outbreaks have occurred since 1994[10]. Outbreaks have persisted despite sanitizing recommendations by the FDA and the increased awareness of the potential for contamination of raw sprouts. Several of the outbreaks summarized in Table 1 trace the contamination of alfalfa sprouts to contaminated seeds. The 1999 *S.* Mbandaka outbreak strengthens the argument for the use of chlorine as a disinfectant. Contaminated sprouts were traced back to a single seed lot that was shipped to sprout growers in California, Washington, and Florida. Out of six sprouting facilities, two did not use any form of disinfectant on the seeds and were linked to the outbreak[10]. However, the effectiveness of chlorine treatments has been questioned, since outbreaks have been linked to seeds that had been disinfected. In 2001, an outbreak of *S.* Kottbus was connected to seed that had been treated with heat and 2,000 ppm of sodium hypochlorite (NaClO)[10]. Also, a *S.* Muenchen outbreak in Wisconsin was linked to seeds that were treated with 20,000 ppm of $Ca(OCl)_2$. Knowledge of the outbreaks due to the consumption of alfalfa sprouts establishes the fact that a problem exists with the current sanitizing procedures.

Pathogen Removal and Treatment Techniques:

Bacteria, whether on sprouts, on equipment, or in the wash water, must be killed or substantially eliminated by effective techniques. While all methods of sanitation of alfalfa seeds were evaluated, the most popular options are summarized in table 2. $Ca(ocl)_2$ is the most common method of treatment and is recommended by the fda for use on seeds in order to reduce populations of *e. Coli* and *salmonella*[11]. While there is no known seed disinfection treatment eliminating 100% of the pathogens without affecting the seed, using 2,000 to 20,000 ppm of $ca(ocl)_2$ effectively reduces the microbial pathogen level[11]. $Ca(ocl)_2$ kills the bacteria by releasing free chlorine. The chlorine disrupts the cell membrane of the bacteria, disabling its ability to function

Table 1. Selected Outbreaks Caused by the Consumption of Alfalfa Sprouts

Year	Pathogen	Location	No. of Cases	Likely Origin	Comments on Treatment Used Prior to Sprouting
1997-1998	*S.* Senftenberg	CA, NV	60	Sprouter /Seed	Inconsistent Cl_2 treatment (Same sprouter as '98 *E.coli* outbreak)[11]
1998	*E. coli* O157:NM	CA, NV	8	Seed	Inconsistent Cl_2 treatment[11]
1998	*S.* Havana/ Cubana	CA, AZ, MD, NM, UT	40	Seed	2,000 ppm Cl_2 for 30 min. then 300 ppm for a few hrs. (Consistency questioned)[11]
1999	*S.* Mbandaka	OR, CS, ID, WA	87	Seed	No disinfectant used[12]
1999	*S.* Muenchen	WI, 6 US states	157	Seed	20,000 ppm $Ca(ClO)_2$[13]
2001	*S.* Kottbus	CA, AZ, CO, NM	32	Seed	Heat + 2,000 ppm NaClO[14]
2003	*S.* Saintpaul	OR, WA	9	Seed	Disinfecting treatment used. All test results for pathogens were negative[15].

Table 2. Techniques for the Disinfection of Alfalfa Seeds

Treatment Method	Advantages	Disadvantages
Calcium Hydroxide[18,19]	• 1% $Ca(OCl)_2$ and 1% $Ca(OH)_2$ gave similar reductions of *Salmonella*	• Corrosive to skin and eyes • Respirators, protective clothing needed • Slightly higher cost then $Ca(OCl)_2$
$Ca(OCl)_2$[18,20,21,22]	• Significant reductions of populations of *E. coli* and *Salmonella* • Inexpensive	• Corrosive to equipment, skin and eyes • Respirators, protective clothing needed
Irradiation[11,22]	• *Salmonella* not found on sprouts treated with 0.5 kGy • Can be used to reduce pathogens on sprouts	• Pathogens detected on treated seeds used to produce sprouts • Dosage to kill pathogens exceeds FDA limits (>5 kGy) and impacts sprouting
Ozone[22,23,24]	• Rapid dissociation to O_2 without by-products • Non-thermal option for sprouts • Kills organisms faster then chlorine	• Treatment of seeds does not significantly reduce populations of *E. coli* • Large capital expense ($3,500) • Unstable, must generate on-site • Not registered by CDPR for direct contact

and to reproduce, thus killing the cell. The efficacy of chlorine compounds depends on the amount of hypochlorous acid present, which is dependent on the ph of the solution, the amount of organic material in the water, and the temperature of the water. Thus, it is desirable to maintain a ph of between 6.0 and 7.5 to ensure adequate hypochlorous acid activity to obtain optimum chlorine activity[12]. $Ca(ocl)_2$ is the preferred disinfectant because few other treatment options produce similar reductions of pathogen levels, and it is inexpensive and easy to handleenvironmental regulatory analysis.

Environmental Regulatory Analysis:

Regulations and Guidelines

The Environmental Protection Agency (EPA) governs the use of pesticides, such as $Ca(OCl)_2$, on raw agricultural commodities while the FDA has the authority over the residue of chemicals remaining on food. $Ca(OCl)_2$ is labeled as a pesticide since it is intended to be a sanitizer to kill pathogens on alfalfa seeds[13]. While alfalfa sprouts are consumed directly by humans, alfalfa seeds are still classified as food according to the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321 (f))[14]. Due to this classification, sprout producers handling both seeds and sprouts are considered food processors and must abide by the FDA's Title 21 of the Federal Code of Regulations. Food facilities are also required by the Act to register under the Public Health Safety and Bioterrorism Preparedness and Response Act in order to protect the public from a terrorist attack on the U.S. food supply[15].

In California, the CDHS enforces the regulations stated in Title 21. Section 110 of Title 21 outlines proper Good Manufacturing Practices (GMPs) that should be implemented and followed in food manufacturing, packing, and storage by food processors. The GMPs help ensure that the processing facility and equipment are maintained in a manner to prevent food contamination. Contaminated food is deemed adulterated if it contains any poisonous substances that threatens human health when consumed[16]. The CDHS inspects facilities for proper GMP training and implementation on average once per year. The FDA will also inspect food facilities in California about once every five to ten years[17]. The FDA has also recommended Good Agricultural Practices (GAPs) under which the seeds should be grown to minimize contamination[18].

The FDA also recommends a food safety program called the Hazard Analysis and Critical Control Point (HACCP) system, which is built on the foundation of successful GMPs. The HACCP focuses on preventing potential hazards for food-borne contamination by applying a scientific method of controls and measurements in all aspects of the food production line. The seven principles of the HACCP call for food facilities to establish necessary operating procedures, sanitation standard operating procedures (SSOP), and recordkeeping methods enabling sprouts to be traced back to the production facility[19]. The FDA recommends that sprouting facilities both sanitize alfalfa seeds before sprouting and test spent rinse water in order to reduce the risk of pathogens on the sprouts. The use of a pesticide for treatment of foods is regulated by both the federal EPA, the California Department of Pesticide Regulation (CDPR) and the California County Agricultural Commissioners (CAC)[30].

Pesticide Registration Process

All pesticides used in the U.S. must be federally registered with the EPA and with state governments. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) governs the use and sale of pesticides in the U.S. In order for the EPA to register a pesticide, the active ingredient must have a food residue tolerance, an exemption from tolerance, or be on the Generally Recognized as Safe (GRAS) list[20]. $Ca(OCl)_2$ is exempt from tolerance on all raw agricultural commodities such as alfalfa seeds, so its use on food is eligible for registration (40 CFR 180.1054).

There are several types of EPA pesticide registrations. A FIFRA, Section 3 registration authorizes full or conditional registration, Section 5 authorizes an experimental use permit, Section 24(c) is used to issue a special local need (SLN) and can be issued in tandem with a state registration, Section 18 authorizes an emergency exemption, and Section 25 is issued by the EPA Administrator to waive registration requirements for a product[25]. These registrations in most cases must not be submitted by the sprout producer but either by a governmental agency or by the company which manufactures $Ca(OCl)_2$.

Currently $Ca(OCl)_2$ is a registered pesticide for certain agricultural and commercial uses, under Section 3 by both the EPA and by the CDPR. The chemical is also registered with the EPA for use to sanitize alfalfa seeds. However, there is no registered use on the label of $Ca(OCl)_2$ products for sanitizing alfalfa seeds in the state of California. While the current use of $Ca(OCl)_2$ in California does occur despite this lack of registration, its use for sanitizing alfalfa seeds is working to prevent contamination, and therefore, no enforcement actions have been taken in recent years[32]. The priority of state enforcement actions depends mostly on environmental impact and available manpower. To apply for an additional use for an existing $Ca(OCl)_2$ product, information such as toxicity data, exposure data, and efficacy data must be submitted to the EPA (40 CFR, FIFRA). A new food use registration for a $Ca(OCl)_2$ to be used indoors would likely take 24 months to process and cost $150,000, but if the product can be shown to have a low "reduced risk" to the public, the process could take 22 months at a cost of $200,000. Federal maintenance fees must also be paid yearly in the amount approximately $60,000. Since most sprouting facilities are classified as small businesses under the FIFRA, these fees can be reduced by 50% to 100%[21].

At the state level, the CDPR believes that data submitted for an EPA registration is not fully sufficient for a California registration. The EPA expects registrants to conduct efficacy studies to support their claims, but waives the actual submission of the information. California asks for this efficacy data to be submitted during the registration process. The state can also request additional data to support Section 24(c) SLN registrations. $Ca(OCl)_2$ has been identified as needing no extensive scientific evaluation, so the processing time for a California new use product registration of 120 days may be much shorter[22].

A public comment period of 30 days follows the processing time before a registration is complete. The proposed registration is posted at CDPR district offices, website, and at CAC offices. If requested by residents or stakeholders, a public hearing may also be held to discuss the impact of the new pesticide on the community. All oral and written comments are published in a report which is reviewed before a final decision is made on the pesticide registration[34,23].

Worker Safety

The Occupational Health and Safety Administration (OSHA) ensures that each employer provides a place of employment free from recognized hazards likely to cause harm to employees[24]. There are several OSHA standards pertaining to injury reporting and industry safety which govern a sprouting facility. For example, OSHA recommends under ideal conditions, industrial workers be limited to lifting 51 pounds[25]. Workers in an alfalfa sprouting facility will constantly be lifting both dry and wet seed bags weighing nearly 50 pounds. However, the seeds absorb water when soaking, so experiments were performed to determine the additional weight of the absorbed water. The seeds were weighed, soaked in water, drained, and then reweighed. The water retained fraction of washed and drained seeds was determined to be 0.24 grams of water per gram of seed compared to the literature value of 0.22 grams of water per gram of seed[18].

In addition, the Material Safety Data Sheet (MSDS) for $Ca(OCl)_2$, or any other chemical, should always be followed and kept in the facility. Employees working with the $Ca(OCl)_2$ must be trained in the chemical hazards and proper handling and storage procedures in the MSDS.

At the state level, the Cal/OSHA program is responsible for enforcing California laws and regulations covering workplace safety by assisting both workers and employers. The enforcement unit conducts inspections based on worker complaints and reported accidents or illnesses. Workers have the right to file a complaint or request an inspection from the Cal/OSHA office.

The Cal/OSHA officers conduct site inspections at least once a year[26]. Workers can also call the county agricultural commissioner regarding pesticide safety issues. Each pesticide user is issued a permit from the CAC office allowing the calcium hypochlorite treatment. The CAC will conduct inspections at least once a year of each site using the pesticide for agricultural purposes[27]. However, since calcium hypochlorite has several non agricultural uses, anyone can purchase and use the chemical without a CAC permit. A facility without a permit most likely will not be inspected unless a public health incident occurs. The CDPR has many similar responsibilities to the Cal/OSHA program along with a Worker Protection Program, which develops outreach materials such as Pesticide Safety Information Series leaflets[28].

Bench Scale Experimentation:

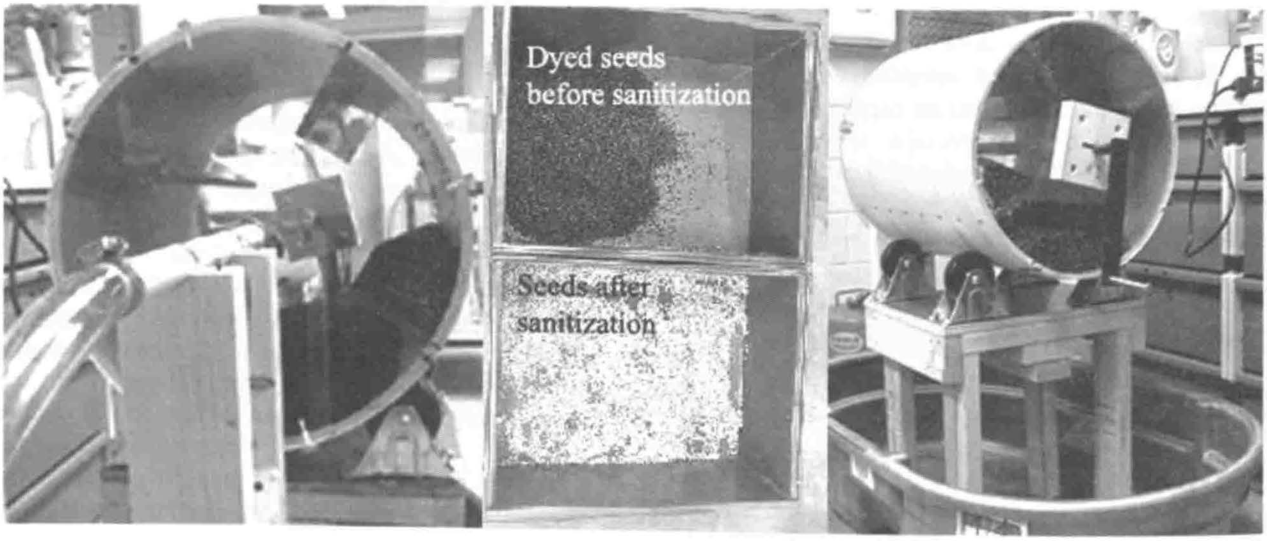
Contact Variation Experiments

A common industry procedure to sanitize alfalfa seeds is to fill a mesh bag with seeds and immerse the bag in a solution of $Ca(OCl)_2$. To determine potential problems with this treatment, lab experimentation that simulated current treatment procedures was performed. The goal of the experiment was to determine whether portions of seeds are contacted with sanitizer solution less effectively than others. In order to detect contact variation, alfalfa seeds were dyed with crystal violet dye until the seeds were darkly colored. 2.2 lbs of seeds were placed into a cheesecloth sack and immersed in a 5000 ppm $Ca(OCl)_2$ solution for 15 minutes with minimal agitation. The concentration of $Ca(OCl)_2$ solution was chosen to ensure removal of some but not all of the dye. The treated seeds were then dried and differences in seed color were noted. It was evident by visual inspection that dye was removed more effectively from seeds on the bottom and outside edges of the bag. Seeds near the top of the bag were noticeably darker than other seeds. The seeds in the middle of the bag were slightly darker than those on the bottom and outside edges. Thus, it was concluded that with minimal agitation, the contact of the $Ca(OCl)_2$ solution with the alfalfa seeds was not uniform and agitation was necessary to ensure that all seeds were adequately contacted with sanitizing solution. Further experiments were performed involving seeds inoculated with a non-pathogenic strain of *E. coli* (strain VR101) in order to compare contact of seeds in a non-agitated bag (10 lbs loading) with contact in a well-agitated vessel. Results showed that seeds in the agitated vessel were contacted more effectively than seeds in the non-agitated bag. The plate count for the non-agitated seeds was more than twice as large as that for the agitated seeds.

Potential Reuse of Sanitizing Solution

Laboratory experiments were performed to determine the ability to recycle the sanitizing solution. After one use, the concentration of the $Ca(OCl)_2$ was depleted to approximately 16,000 ppm in 15 minutes, which is in agreement with the literature[21]. Therefore, if an effective free chlorine test method is developed, the sanitizer solution could be recycled with the addition of $Ca(OCl)_2$ to bring the solution concentration back to 20,000 ppm. However, since there is not an effective test method for free chlorine concentration, recycling the sanitizer solution is not currently applicable.

Figure 2. Rotary Drum Bench Scale Photograph



Rotary Drum Bench Scale Design

A rotary drum bench scale apparatus was constructed which is shown in Figure 2. The majority of medium to large sprout producers that own a rotating drum use it primarily for germination[29]. The bench scale model was tested for sanitation effectiveness. The purpose was to determine if alfalfa seed can effectively be sanitized in a rotary drum. In experimentation, four pounds of dyed alfalfa seeds were mixed with 0.59 lbs of dry $Ca(OCl)_2$ and placed in the rotating drum. 2.4 gallons (6 gal water per 10 lbs seeds) of water were sprayed into the drum at 15 min intervals for a total of 21 minutes. The drum was rotated, by hand, at approximately 3 rpm to ensure effective contacting between seeds and the $Ca(OCl)_2$. The rotation speed was chosen to ensure sufficient time for contact between the seeds and the $Ca(OCl)_2$. After the cycle, the seeds were found to be free of dye (See middle photograph of Figure 2). Therefore, the rotary drum is recommended, for both sanitation and germination, for current owners of rotary drums or producers able to afford the $14,000 capital cost[30]. If used for sanitizing, the rotary drum should be constructed of material able to withstand corrosion from exposure to chlorine. Testing of the runoff water 48 hours into the germination process as recommended by the FDA is still required with this design[11].

Agitation-in-Bag Bench Scale Design

The agitation-in-bag bench scale apparatus, shown in Figure 3, which was tested using 10 lbs of seed, was constructed to improve the current repetitive-dunking bag procedure used by some small scale sprout producers. For sprout producers currently processing more than 10 lbs of seed in a cycle, the design could be scaled to address the demand. The equipment consists of (1) a 15 gallon mesh drawstring bag, (2) a four-legged cage within the bag, (3) an agitator support plate, (4) an agitator impeller, (5) an agitator drive motor, (6) a 11" diameter by 20 ý" tall [10.5 gallon] sanitizing vessel, (7) a 19 gallon plastic rinsing container, and (8) a 16" by 16" perforated bag support plate for rinsing. The cage keeps the bag from collapsing and provides agitation baffling; it is constructed from ý" PVC pipe and pipe fittings. The agitator impeller is a hurricane paint mixer available at most hardware stores. A ý" variable-speed drill motor is used as the agitator drive motor. For the experimental unit, the drill support plate is constructed from æ" plywood; however, a æ" polyethylene sheet is recommended for long-term use to withstand handling and chemical exposure. The drill is held in a 2" diameter hole in the support plate by two vertical all-thread rods, bolted firmly to the support plate. The cost required to implement the apparatus in practice is given in Table 3.

The itemized steps for the sanitizing procedure are the following:

(1) Put on all personal protective equipment

(2) Insert the cage into the bag along with 10 pounds of seed.

(3) To clean seeds, place bag on grate atop the rinsing container.

(4) Using hose spray seed for about 15 minutes or until run-off water is clear.

(5) Add 6 gallons of premixed 20,000 ppm of $Ca(OCl)_2$ solution to the sanitizing vessel.

(6) Insert bag with cage and seed into the sanitizing vessel.

(7) Pull the bag drawstrings tightly over the cage, leaving space for the impeller.

(8) Insert the impeller [with its shaft firmly in the drill chuck] into the bag.

(9) Start the drill motor at a predetermined speed of about 400 rpm.

(10) Agitate the seeds for 15 minutes.

Table 3. Agitation in Bag Bench Scale Capital Estimate

Item (quantity)	Cost/Item	Total Cost
½" PVC tees & caps (4each)	$0.18	$1.44
½" PVC 45° elbows (8)	$0.16	$1.28
½" Sch. 40 10' PVC pipe	$1.19	$1.19
½" variable speed drill	$47.97	$47.97
1 3/8" all-thread rod	$0.87	$0.87
16" x 16" Return Air Grille	$7.97	$7.97
40 liter cylindrical trash can	$6.94	$6.94
19 gallon plastic tub with rope handles	$4.94	$4.94
¾" 2' x 4' polyethylene sheet	$20.80	$20.80
Warner 5 gal. hurricane mixer	$7.92	$7.92
Miscellaneous (nuts, bolts, etc.)	$1.00	$1.00
Tax at 10%		$10.23
Total		**$112.55**

(11) Stop the agitator and remove the agitation assembly from the sanitizing vessel.

(12) Place the bag and cage on the grate atop the rinsing container

(13) Spray water into the seed bed until odor is undetectable and rinse water runs clear.

(14) Remove the cage and sanitarily transport the seeds to germination.

(15) Dispose of remaining solution to the sewer and disassemble apparatus for cleaning.

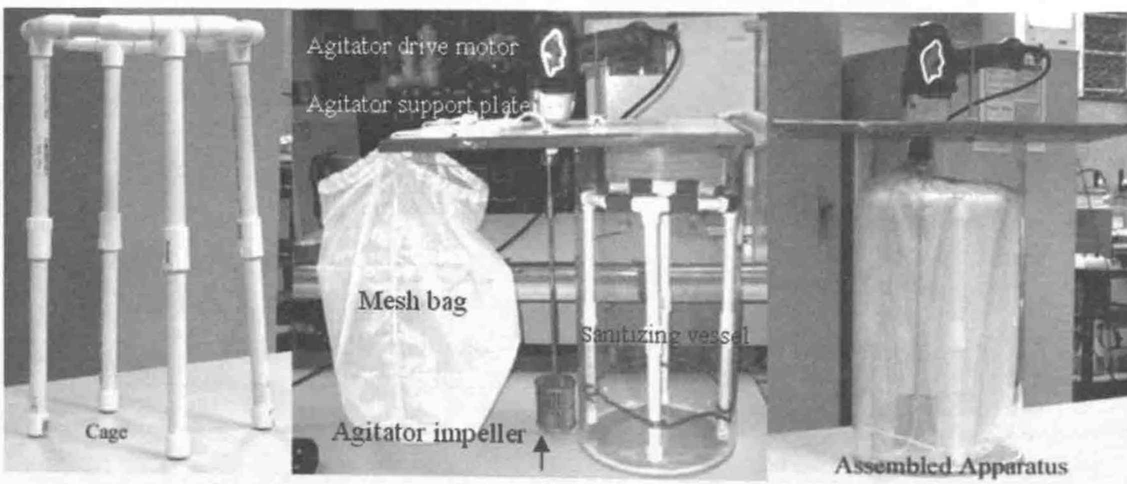
The effectiveness of the agitation in bag system was tested experimentally. Alfalfa seeds were dyed with crystal violet dye. After agitating the seeds at 370 rpm for 15 minutes in a solution of 5000 ppm $Ca(OCl)_2$, visual inspection indicated uniform dye removal. The alfalfa seeds were allowed to sprout. Essentially all the seeds germinated, indicating the agitation did not significantly effect sprouting efficacy.

through the PVC tube. The seed feed rate was 4.5 lbs/hr with a sanitizing solution (20,000 ppm $Ca(OCl)_2$) feed rate of [(6/10)(4.5)/60] = 0.05 GPM to give a 5:1 ratio of sanitizing solution to seed feed rate. The auger speed required was 3.5 rpm to give a seed residence time of 15 minutes. The PVC tube was elevated at an angle of 15 degrees. The sanitized seeds are conveyed from the upper end of the PVC tube onto the vibrating screen.

The screen is vibrated moving the seeds toward the collection bin. As the seeds are conveyed along the vibrating screen, rinse water is sprayed uniformly along the length of the screen onto the seeds. A rinse water collection bin is placed underneath the screen to collect the rinse water and sanitizing solution. The rinsing headers are two æ" ID, 24" long PVC pipes with 3 rinsing nozzles each. The headers are supplied by the rinse water tank and a ý HP centrifugal pump. A 5 gallon collection bin is located at the end of the separator to collect the sanitized and rinsed seeds.

Experiments were conducted to determine the effectiveness of the auger system. Dyed seeds were fed at 4.5 lb/hr with 20,000

Figure 3. Agitation in Bag Bench Scale Photograph



ppm $Ca(OCl)_2$ solution through the auger. The auger was operated at 3.5 rpm for 45 minutes. The dye was effectively removed from the seeds during the sanitation process.

Auger Bench Scale Design

The auger bench scale experimental apparatus, shown in Figure 4 and Figure 5, was constructed to model the one ton per hour system. Each stream in the figure is numbered corresponding to the stream attributes table, which lists the stream compositions. The apparatus consists of a sanitizing unit and a rinsing unit. The sanitizing unit consists of: (1) a 1.5" ID x 4' long transparent PVC tube, (2) a 1.375" OD x 4' long SS auger, (3) a 5.5" x 6" x 15" tall feed bin, and (4) a 1/8 HP systolic pump. The rinsing unit consists of: (5) a vibrating screen, (6) two rinse water headers, (7) a rinse water pump, (8) a rinse water tank, (9) a seed collection bin, and (10) a rinse water collection bin.

The seed and sanitizing solution are supplied to the feed bin. The auger conveys the seeds and the sanitizing solution

The bench scale washer was a 6" wide by 30" long screen stretched tautly over a wooden frame, which was supported on springs to allow the screen to be vibrated by tapping on the frame by a hammer. Odor tests on the seeds and seed-exit rinse water samples indicated effective $Ca(OCl)_2$ removal.

Full Scale Design:

The full scale design, shown in Figure 6, was sized based on the bench scale unit. Each stream in the figure is numbered

Figure 4. Auger Bench Scale Photograph

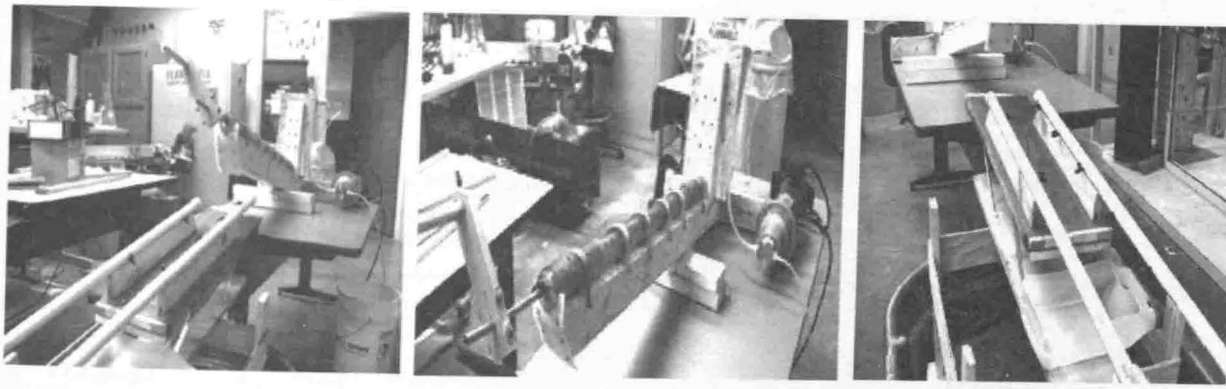
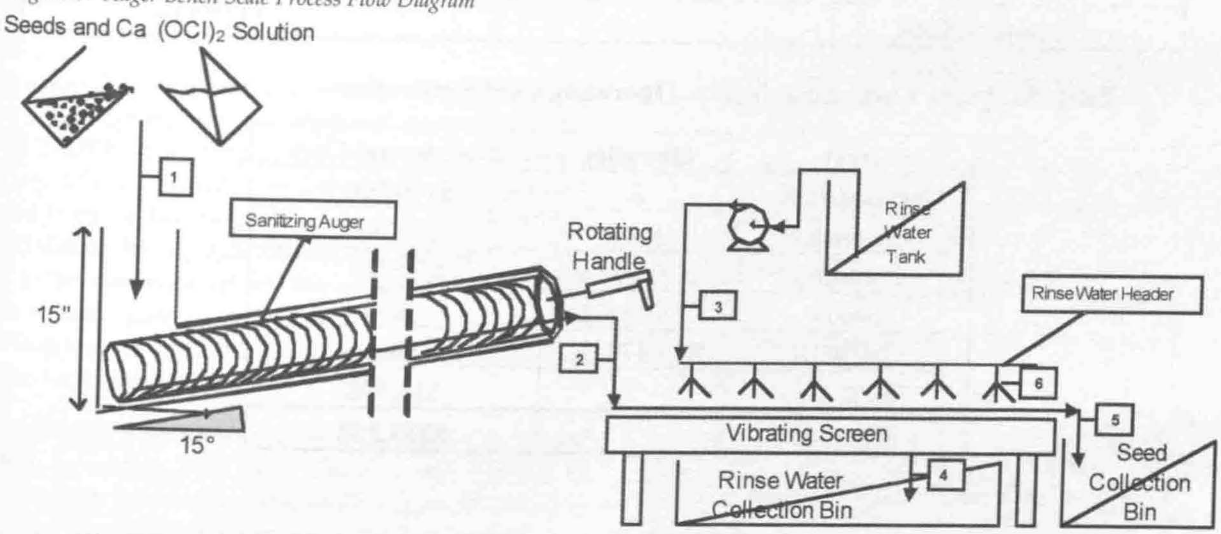


Figure 5. Auger Bench Scale Process Flow Diagram



Bench Scale Stream Attributes						
	1	2	3	4	5	6
Temperature, °F	75	75	75	75	75	75
Pressure, psig	0	0	0	0	0	0
Alfalfa Seed, lb/hr	4.5	4.5	0	0	4.5	0
Ca(OCl)2 Solution, gpm	0.045	0.01	0	0.01	0	0
Rinse Water, GPM	0	0	0.9	0.8975	0.0025	0.15
Organic Solids/dirt, lb/ft³	trace	trace	0	trace	0	0

corresponding to the stream attributes table, which lists the composition of the streams. Calculations were performed to achieve a seed feed rate of one ton per hour. However, any seed feed rate ranging from 4.5 to 2000 lb/hr can be used for the auger system. The bench scale unit was operated at a seed feed rate of 4.5 lb/hr. The full scale unit requires a feed rate of 2000 lb/hr; thus, the scale-up factor is 2000/4.5 = 444. The bench scale unit had an auger volume of 0.05 ft^3; thus the plant auger must have a volume of 444(0.05) = 22 ft^3. With a 16" diameter auger the required auger length is [22/(4/þ)(16/12)^2] = 18'.

The full scale unit capacity is 5.6 million lbs/yr based on one eight hour shift per day, 350 days/yr. The unit has the potential to process 17 million lbs/yr if run 24 hrs/day, 350 days/yr. The capital cost of all equipment is listed in Table 4. Calculations for the operating costs, listed in Table 5, of the auger system were taken on an incremental basis from an existing facility.

The full scale unit operates in four stages: solids loading, sanitizing solution preparation, sanitizing, and rinsing. The

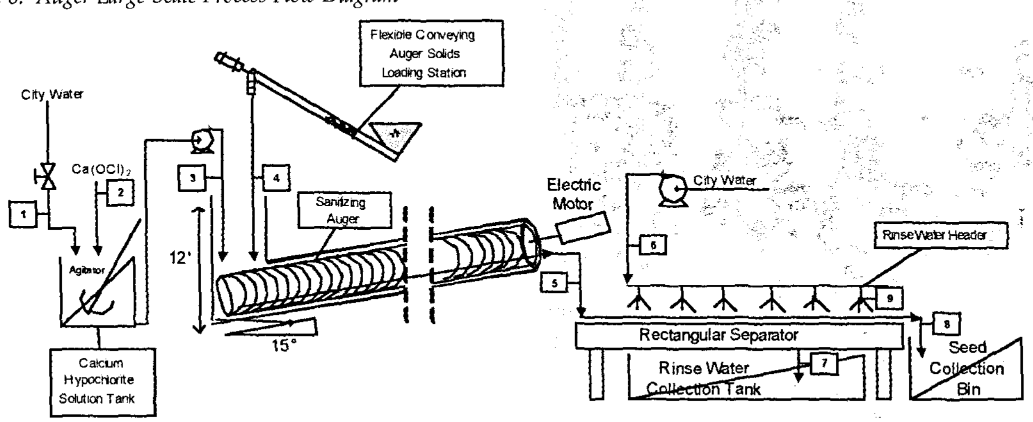
Table 4. Auger Large Scale Capital Cost Economics[43]

Equipment(Quantity)	Description	Purchased Cost	Installed Cost
Sanitizer Unit	Feed bin, auger, housing motor	$18,956	$31,278
Rectangular Separator	Sprayer, water collection trough	$51,000	$84,150
Flexible Auger	0.75 HP, 20' high screw conveyor	$18,350	$30,278
Ca(OCl)2 Tank	16,500 gallon Vertical Poly Tank	$15,000	$24,750
Agitator	Turbine Agitator	$20,000	$33,000
Solution Pump	Centrifugal Pump, 0.75 HP	$252	$416
Seed Collection Tub (8)	100 gallon Polyethylene Tub	$1,576	$2,600
Total (14)		**$125,134**	**$206,471**
Tax at 10%		**$20,647**	
Total Capital Cost		**$227,119**	

Table 5. Auger Large Scale Yearly Operating Cost Economics

Item	Quantity	Incremental Cost
Operator Salary	3	$180,000
Electricity	10 kW	$1,260
Water	8,400,000 gal	$12,720
Seeds	5,700,000 lbs	$0
$Ca(OCl)_2$	855,470 lbs	$0
Tax at 10%		$19,398
Total		**$213,378**

Figure 6. Auger Large Scale Process Flow Diagram



Full Scale Stream Attributes									
	1	2	3	4	5	6	7	8	9
Temperature, °F	75	75	75	75	75	75	75	75	75
Pressure, psig	40	0	0	0	0	40	0	0	40
Alfalfa Seed, lb/hr	0	0	0	2000	2000	0	0	2000	0
$Ca(OCl)_2$, lb/hr	0	300	300	0	60	0	60	0	0
Water, GPM	20	0	20	0	5	10	14	1	0.48
Organic Solids/dirt, lb/ft³	0	0	0	trace	trace	0	trace	0	0

solids loading stage consists of (1) a flexible conveying auger solids loading station. The sanitizing solution preparation stage consists of: (2) 16,500 gallon vertical poly sanitizing solution tank, (3) a turbine agitator, and (4) a 30 GPM (20' head) noryl centrifugal pump. The sanitizing stage consists of: (5) an auger tube with a feed bin at the inlet end, (6) a 12" OD x 18' 304SS auger with a Pitch/Diameter of 1/2, and (7) a 1.5 HP 3-5 rpm variable speed drive. The rinsing stage consists of: (8) a universal motion rectangular 3' wide by 15' long vibrating screen separator and (9) a 1.5 HP variable speed (30 rpm max.) motor. The rinse water is sprayed onto the screen by (10) about 21 square pattern spray nozzles attached to PVC headers. The rinsing stage also consists of: a (11) rinse water collection trough underneath the vibrating screen to pump the water to the sewer and (12) sixteen, 100 gallon polyethylene seed collection wheeled tubs.

The solids loading process begins by manually loading seed from 55 lb bags into the feed hopper of the seed conveyor at a rate of 2,000 lb/hr, or roughly one bag every two minutes. One operator will be required to load the seeds. The seeds will be removed from the bag by placing a bag on the feed hopper grating and slitting the bag with a box cutter. Before the seed is conveyed to the sanitizer feed bin, the seeds are washed inside the hopper removing any loose debris. The seeds are elevated from the feed hopper to the sanitizer feed bin by the flexible auger of the feed conveyor.

The sanitizing solution is prepared on a batch basis, for one 8 hour shift, by adding 2400 lb of $Ca(OCl)_2$ and 120,000 lb (14,400 gal) of city water to the 15,000 gallon agitated sanitizing solution tank. The sanitizing solution is fed into the sanitizer feed bin by a noryl centrifugal pump at 20 GPM and 30 feet of head. The inclined (15×) sanitizing auger rotates at about a rate of 3-5 rpm. The auger conveys the seeds and the sanitizing solution. The inclination and auger speed were determined to give a residence time of 15 minutes for the seeds in the auger. A 15 minute contact time is recommended by FDA for $Ca(OCl)_2$ treatment.

The seeds are discharged from the upper end of the sanitizer, and fall onto the rectangular separator. As the seeds are conveyed and agitated by the separator, rinse water is sprayed through 21 square pattern nozzles at a rate of 20 GPM. The rinse water and chemical residue drains from the seeds through the separator into the rinse water collection tank. The waste water is taken from the rinse water collection tank and disposed of into the sewer. At the end of the separator, the sanitized and rinsed seeds drop into wheeled seed collection bins. At a 500 lb capacity for the bins, a bin will be filled every 15 minutes. The seed collection bins are then transported to the sprouting area, which will require one full time operator. A third full time operator will be required to handle all other aspects of the process except for seed loading and seed transportation from washing to sprouting.

Business Plan:

The business plan for the IMPACT project involves the possibility of two different processes, one available for the large volume sprout producer and one for the small volume sprout producer. Both processes can be integrated into existing post-harvest operations or into a new post-harvest facility. The process for the large volume sprout producer consists of an auger/vibrating screen system. The project can be completed in about 22 weeks. Six weeks are required for design, 10 weeks for equipment delivery, and 6 weeks for construction and start-up. The operating cost for the auger system capable of processing a ton per hour of seeds is \$214,000. The total installed capital cost of the system is \$227,000.

Alternately, the business plan involves the installation of the agitation-in-bag method for the small scale sprout producer. The project to implement this method can be completed in about one week. All the materials except the polyethylene mounting board can be purchased at local hardware stores. Two days are necessary, however, if special ordering for equipment is needed. All of the equipment can be assembled and installed in about two days with the remainder of the week necessary for startup, testing and training. There are no additional operating costs for the implementation of the design. The total capital cost of the bag agitation system is \$113.

The research work for this investigation showed a substantial improvement in pathogen kill by using the agitation-in-bag method rather than the hand-dunking method. Considering the modest capital investment and no additional operating manpower, the improvement in contacting effectiveness warrants immediate implementation of the agitation-in-bag sanitation procedure.

Conclusions and Recommendations:

Existing literature and research shows that $Ca(OCl)_2$ is an effective treatment for the pathogens found to contaminate alfalfa seeds. Experiments have shown problems exist with current practices concerning contact of sanitizer and seeds. Through experimentation, it was determined the rotary drum can effectively sanitize and germinate alfalfa seeds. Therefore it is recommended for the sprout producers that currently own a rotary drum, to use the drums for both sanitizing and germination. The agitation-in-bag method is a viable and economically attractive option for small volume sprout producers, up to about 80,000 lbs of seeds per year. The cost-effective design improves a current industry practice by minimizing pathogen contamination of seeds by providing very effective contacting of seeds and sanitizing solution.

The auger/vibrating screen system is technically viable for any production rate and could be used by small producers;

however, it is mostly applicable for partnerships of sprout growers with seed requirements greater than 80,000 pounds per year. The auger/vibrating screen system is a relatively modest investment for large producers compared to sanitizing large seed volumes by hand.

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Faculty Comment:

Roy Penney, in submitting this article for publication consideration, made the following remarks about the work:

> The seven authors of this paper are all senior Chemical Engineering students. They participated as a team [the IMPACT (IMProved Alfalafa deContamination Techonologies) team] in Task 2 of

the 2005 WERC [Waste-management, Education and Research Consortium, WERC.net] Environmental Design. The IMPACT team started work on Task 2 during Christmas Break in early January 2005 and finished the competition at NEW Mexico State University (NMSU) April 4-7, 2005. The Task 3 problem statement was: "*Develop a simple and practical system to use calcium hypochlorite (sanitizer) to treat sprout seeds. The solution must be cost effective and take into account all aspects of chemical treatment (e.g., storage, handling, waste, etc.)*". All team members received 3 hours of credit for CHEG 4443, Senior Chemical Engineering Design II.

There have been repeated outbreaks of food poisoning from contaminated alfalfa sprouts, especially in the US Northwest. The USDA believes that the contamination originates from alfalfa seeds contaminated with Salmonella and e-Coli. The IMPACT team did a thorough analysis of the US sprouting industry (which included a thoroughly-documented visit to a Kansas sprout producer) and determined that the most likely cause of seed contamination was inadequate washing of seeds with Calcium Hypochlorite solution by the current hand dunking method. IMPACT developed a simple cost-effective (capital cost•H $100) mechanical agitation system which effectively treats seeds and remove all pathogens, as demonstrated in laboratory experiments at the University of Arkansas and at NMSU.

The team also developed a cost effective process for treating up to one ton/hour of alfalfa seeds to satisfy a stated task requirement— "*address a seed feed rate of one kilogram per hour with a **maximum of one ton per hour***". Although, no single sprout producer in the US currently processes anywhere near 1 ton/hour of seeds, the SWARE team developed a cost effective process to handle that quantity at that speed. Seed disinfecting with hypochlorite solution was done effectively in a continuous auger unit and the final water washing was accomplished on a vibrating, sprayed screen. The one ton/hour IMPACT process was demonstrated in laboratory experiments at the University of Arkansas and at NMSU.

The IMPACT team performed very well by (1) determining the demographics and operating practices of the US alfalfa sprout industry, (2) developing, and experimentally demonstrating, an economical mechanical-agitation alternative to the current hand dunking method, and (3) developing a cost-effective process for treating up to 1 ton/hour of seeds, which, also was experimentally demonstrated. Their excellent performance was rewarded with a first place award in task and a USDA-CSREES Award of Excellence. The USDA-CSREES award includes a trip to Washington, D.C. for the team and its advisor to present their work to the USDA and other government agencies.