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*Article*

# Annualization of the Long Day Onion Breeding Cycle through Threshold Vernalization and Dormancy Disruption

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## ABSTRACT

*Background*: Genetic gain is a function of several parameters including generation time. Onion is a biennial crop that typically requires two full calendar years per generation or breeding cycle. Annualization of onion breeding could double the rate of genetic gain but is technically difficult because of bulb vernalization and dormancy requirements.

*Methods*: Based on recent results that demonstrate vernalization thresholds for long-day onion, we conducted an experiment to assess whether shorter vernalization times could result in faster, uniform flowering with dormancy-broken onion bulbs. Bulbs from eight varieties were subjected to a 15% hydrogen peroxide treatment for 4 h in order to break dormancy. This treatment was followed by either 6 or 12 weeks of vernalization after the first physical signs of dormancy release. The vernalized plants were transferred to 16-h daylengths in a 20 °C greenhouse for flowering.

*Results*: The treatments imposed in this study resulted in the completion of a full generation in less than 12 months for all eight varieties examined. As the length of vernalization at 10 °C increased, we observed greater uniformity in floral initiation and a higher proportion of scape emergence. Plants that were vernalized for 6 weeks flowered 24% earlier than those in the 12-week treatment group. However, we observed greater uniformity in scape emergence both within and among varieties when vernalized for 12 weeks.

*Conclusions*: We have developed a framework for an annual cycle breeding system that offers the ability to phenotype bulb traits. We have also identified aspects of the system that will benefit from further refinements. Overall, this annualization system may be useful for advancing generations in onion breeding and reducing the time required to produce new varieties.

**KEYWORDS:** *Allium cepa* L.; annual cycle; dormancy; vernalization; flowering; bulb; long-day; breeding



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## INTRODUCTION

Genetic gain is predicated on trait heritability, selection intensity, phenotypic variance, and the duration of the breeding cycle. Some annual crop plants, such as bread wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oats (*Avena sativa*), and various *Brassica* species can be bred on a shortened cycle [1–3], and in certain cases multiple generations can be accomplished in a single calendar year. Shortened cycle, or "speed breeding", techniques manipulate environmental conditions, such as temperature and light, to promote early flowering and seed development [2]. Genetic gain in onion (*Allium cepa*), a biennial crop, is hampered by a two-year breeding cycle and would benefit from a robust speed breeding protocol. Developing an annual cycle breeding scheme for onion has been a topic of conversation in the research community since at least 1945 [4], and is emblematic of both the fundamental challenge of working with biennial species and the desire to improve breeding efficiency in this crop.

Some biennial vegetables can be bred on an annual cycle through the use of controlled environment production systems, such as a greenhouse. Carrot (*Daucus carota*) and beet (*Beta vulgaris)* fall into this category, and can complete a single generation in one calendar year provided such controlled environment systems are available [5,6]. However, onion has a two-year generation time because of the length of time necessary to complete each stage in the plant's life cycle combined with our lack of understanding of the conditions required for optimum floral initiation and development. In a typical long-day onion seed production system, seed is sown in the spring to produce bulbs by autumn. The bulbs are harvested, overwintered in cold storage, or left in the field in temperate environments, and replanted in the spring of the following year [7]. Bulbs are often held in storage for up to 7 months, until the weather is warm enough for planting. Once in the ground, the bulbs sprout, flower by early summer, and produce seed over the next one to 2 months. While the overall timeframe from seed-to-seed is less than 24 months, the practical reality is that two full growing seasons are required in a temperate climate. This two-year generation time is challenging for plant breeders and seed producers because it results in half the gain from selection per annum of a comparable annual crop species and lengthens the time from an initial cross to varietal release. It also serves as a barrier to entry for onion genetic research, which often relies on project specific population development. Due to the time needed to reach advanced generations, population development requires considerable foresight and careful planning. Many of these issues would be alleviated through the implementation of an annualization procedure that would allow for completion of the full onion life cycle within a calendar year.

For an annual cycle system to be a useful tool for breeders and other researchers, it should meet certain criteria. Principally, the technique must offer the ability to go from seed to seed in under one calendar year. Ideally, if seed were harvested by the 11th month following planting,

enough time would remain to clean and process seed prior to planting for the next growing season. This timeline would also build in buffer time to account for year-to-year variation in seed harvest date or planting date. To this end, the annualization procedure needs to be repeatable, reliable, and consistent in its performance across both years and environments. Any annualization strategy will require a greater investment of time, energy, and resources than current biennial breeding practices, with the payoff being the ability to cut the generation time in half and thereby increase the potential for genetic gain.

One major concern for annualization of biennial crops is indirect selection for bolting susceptibility. By creating a selection environment that favors bolting and rapid flowering, there is a risk of inadvertently selecting plants that flower more readily, which could result in poor bolting tolerance during bulb production [7,8]. This is a valid concern and one that could have costly consequences for both farmers and seed companies. To avoid selecting for bolting susceptibility, the vernalization treatment should be similar in length to the optimum vernalization time, which is the minimum duration of chilling required for uniform floral initiation, for commercial varieties with proven bolting tolerance. The optimum vernalization time differs from the optimum storage duration in that the optimum vernalization time references chilling received after endodormancy release. This is a vital distinction because sprouted and unsprouted bulbs have been found to vernalize at different rates, which suggests that bulb dormancy status affects the response to vernalization [9,10]. Our goal for annualization is not to eliminate vernalization but rather use it as a tool to ensure fast, uniform flowering across diverse germplasm while leaving opportunities to screen for bolting tolerance intact.

Another important consideration for an annualization system is that it should offer opportunities to phenotype traits of interest. These include traits related to growth habit, pest and disease resistance, skin color, bulb shape, single centeredness, vigor, bolting susceptibility, flavor, and storability, to name a few. An annual cycle breeding system should be able to consistently operate across a range of genotypes and environments with little year-to-year variation. This requires flexibility to be built into the design of the annualization system to accommodate differences in optimal conditions at each stage of growth, especially for floral initiation.

Current strategies in onion annualization leverage the seedling vernalization technique, which induces flowering through vernalization of seedling plants prior to bulbing [11]. This is an annual cycle seed to seed system that sidesteps bulbing and the onset of endodormancy through careful control of environmental conditions to force flowering during the first season of growth [4,12]. Seedling vernalization offers the opportunity for annual cycle breeding but is limited in that it does not allow for phenotypic evaluation of most bulb traits [13].

We previously determined that long day onion varieties can achieve uniform scape emergence with an optimum chilling time of 12 to 14 weeks at 10 °C [14]. Through the course of this work we identified and quantified an endodormancy period that is present in the bulbs of these varieties and lasts for 8 to 10 weeks [15]. This finding indicated that the bulbs were endodormant for more than half of the 14-week optimum chilling time. Utilizing these findings, we demonstrated that a 2 to 4-h exogenous treatment of 15–20% hydrogen peroxide was highly effective at initiating uniform root growth in endodormant bulbs, thus signifying the release from endodormancy [14]. Bulbs treated in this way could then be subjected to precise lengths of vernalization, thereby reducing the overall time to flowering.

To our knowledge, there are no published descriptions of an annual cycle breeding strategy that includes the production of mature bulbs. By taking advantage of the seven-month period between bulb harvest and planting, a second growing season can take place in a controlled environment. If grown under the correct conditions in a greenhouse, dormancy-released bulbs can flower, be pollinated, and produce viable seed in time for sowing in the field during the following season. Through this work, we sought to identify the conditions and treatments necessary for a successful annual cycle breeding system in long-day storage onions. The experiment described herein was conducted as a proof of concept of an annualized onion breeding system that includes an opportunity to phenotype bulb traits. The creation of an effective, reliable annualization procedure would take years off varietal development in onion breeding programs and provide researchers with a new set of tools to conduct experiments.

## MATERIALS AND METHODS

*Field design.* Onions were grown under commercial growing conditions on muck soil at Jack's Pride Farm in Randolph, WI, USA. Seed was sown on May 4, 2017 into raised beds using a modified Planet Junior planter equipped with a cone seeder attachment. Plots were planted in 3.66-meter rows with a 1.22-meter alley and 30 cm row spacing. Eight accessions, which are representative of the material used in our breeding program, were evaluated in this experiment: W460C, W419B, CUDH2107, WEOS1, W205A, W455A, "Cortland", and "Sherman". W460C, W419B, W205A, and W455A are inbred lines developed in the University of Wisconsin onion breeding program [16]. W205 is pink root resistant and has moderate fusarium resistance. It was derived from inbred lines C72 and B2215. W419 is resistant to pink root and fusarium and was derived from the inbred lines W101 and W205. W455 possesses some resistance to white rot and onion maggot but is susceptible to pink root and fusarium. It was derived from W404 and PI2646540. W460 has resistance to white rot and fusarium but is susceptible to pink root. It was derived from crosses between W404 and PI2646540. WEOS1 is an open-pollinated synthetic

population selected through recurrent selection for earliness and released by the University of Wisconsin onion breeding program. It was derived through a recurrent selection program comprised of early maturing long day onion germplasm. CUDH2107 is a long-day storage doubled haploid line produced at Cornell University [17], and “Cortland” and “Sherman” are $F_1$ hybrid cultivars from Bejo Seeds. Each variety was grown as a single field replication except CUDH2107 which included two replications. The bulbs were harvested on September 18, 2017 and were cured at ambient temperature in a dark, well-ventilated storage room for nine days prior to treatment.

*Chemical source*. A 30% solution of stabilized hydrogen peroxide ($H_2O_2$) (VWR International, BDH7690-1, Radnor, Pennsylvania, USA) was used as a chemical stock during the dormancy release phase of this experiment. This stock solution used sodium stannate ($H_6Na_2O_6Sn$) as a stabilizer. The 30% solution was diluted to a final concentration of 15% using purified water (Thermo Scientific E-pure Water Purification, Model 7117, Waltham, Massachusetts, USA) immediately prior to treating the dormant bulbs.

*Bulb treatment*. Fifteen bulbs were sampled from each variety and treated with hydrogen peroxide to force the release of bulb endodormancy. We have previously demonstrated that hydrogen peroxide can break bulb endodormancy when treated with 10–30% solutions for 2 to 4 h [14]. Due to a hail storm late in the season, we were unable to sample 15 bulbs from 4 of the varieties: W419B, W205A, W455A, and “Cortland”. No fewer than 10 bulbs were used from each of these varieties. The top third of each bulb was cut so that the basal plate and 2/3 of the bulb remained. In bulbs with early symptoms of center rot, additional bulb tissue was cut off to remove additional sources of inoculum. Bulbs were placed into a 11.3 L plastic container (26.2 × 40.1 × 17.8 cm) with the cut side facing down while 350 mL of a 15% hydrogen peroxide solution (weight by volume) was added to the container (Figure 1). The bulbs were left to soak in covered plastic containers at ambient temperature for 4 h. Upon completion of the treatment, the bulbs were removed from the container and rinsed under cool tap water to remove any excess solution. The bulbs were then planted in a 20 °C greenhouse under a 16-h daylength in a completely randomized design. After 3 weeks, 2/3 of the bulbs from each variety were transferred to a 10 °C temperature-controlled growth chamber with fluorescent lighting under 16 h of daylength and a photon flux density of 10.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Onset Computer Corporation, HOBO UA002-64, Bourne, Massachusetts, USA). Once transferred to the growth chamber, the bulbs were randomized on the shelves. The remaining bulbs were kept in the 20 °C greenhouse as a non-vernalized control. The plants that were transferred to the growth chamber were divided into two treatment groups based on their intended vernalization time. One group remained in the growth chamber at vernalizing temperatures for 6 weeks, while the other was vernalized for 12 weeks. At the end of each treatment group’s respective vernalization

period, the plants were transferred back into the 20 °C greenhouse and randomized on the bench.

**Figure 1.** Cut bulbs soaking in 350 mL of 15% hydrogen peroxide solution.

*Greenhouse culture*. All bulbs were planted in 2.78 L nursery pots. The potting media was a 2:1 mix of silty loam compost soil collected from the West Madison Agricultural Research Station and soilless medium (MetroMix; Sun Gro Horticulture, Agawam, MA, USA). An air-conditioned greenhouse with forced air heating was used to maintain a temperature of 20 °C. A 16-h daylength was maintained using high pressure sodium supplemental lighting. Plants were watered daily and were fertilized sparingly with a 400-ppm mixture of 20-10-20 fertilizer with micronutrients.

*Data collection and analysis*. Bulbs were monitored every two to three days for signs of root development, sprouting, and scape emergence. These data were collected throughout all stages of this experiment, including when the plants were in the growth chamber. Rooting was defined as the presence of a white, actively growing root originating from the bulb basal plate. Individual plants were visually inspected for root initiation by lifting the planted bulb from the pot. Following visual inspection, the bulb was replaced in the medium. Sprouting was defined as the presence of green leaf tissue emerging from the center of the cut surface of the bulb. Scape emergence was defined as the presence of the spathe from a leaf sheath at the crown of the plant. The date that each event was first observed was recorded on an individual plant basis and used to calculate the time to each event relative to planting date and the date each treatment group was removed from the growth chamber. Percent scape emergence was calculated as a percentage of sprouted bulbs that went on to develop scapes. Bulbs that never sprouted were not included in this percentage.

Tukey's honest significant difference (HSD) test was performed on scape emergence data using the "stats" package in R [18].

## RESULTS

The treatments imposed in this study resulted in the completion of a full breeding cycle in less than 12 months for all eight varieties examined. However, length of vernalization time and genetic background were important factors in the overall length of the annual cycle and the uniformity of flowering.

As the length of vernalization at 10 °C increased, we observed greater uniformity in floral initiation and a higher proportion of scape emergence (Figure 2A). On average, plants that were vernalized for 6 weeks produced scapes 24% earlier (approximately 25 days) than those in the 12-week treatment group (Tables 1 and 2). However, we observed greater uniformity in scape emergence both within and among varieties when vernalized for 12 weeks (Figures 2B and 3B). We found a highly significant reduction in time to scape emergence for the 6-week treatment group, but there was not a significant difference between the 0 and 12-week groups (Table 2). Scape emergence and flowering were observed in all varieties for the non-vernalized control except in W455A. Although scape emergence in the non-vernalized control was coincident with plants from the 12-week treatment, the proportion of plants that produced scapes in the control group was lower for all varieties. As vernalization time increased, the proportion of plants that produced scapes also increased (Figure 2A), the notable exception being CUDH2107 (Table 1). There were two field replications for CUDH2107, in one replication 100% of the plants from the control group produced scapes while we only observed scape emergence in 40% of the plants in the 6 and 12-week vernalization treatments. The other field replication performed similarly to the other varieties evaluated in that the proportion of plants with scapes increased with vernalization time.

**Table 1.** Percent scape emergence and mean days to scape emergence by variety across each of the three vernalization treatment groups. Note the trend that percent scape emergence increases in tandem with vernalization time.

| Variety | % Scape Emergence | | | Mean Days to Scape Emergence | | |
|---|---|---|---|---|---|---|
| | 0 Weeks | 6 Weeks | 12 Weeks | 0 Weeks | 6 Weeks | 12 Weeks |
| Cortland | 67 | 100 | 100 | 174 | 112 | 147 |
| CUDH2107 | 60 | 33 | 78 | 144 | 158 | 167 |
| Sherman | 60 | 100 | 100 | 149 | 108 | 142 |
| W205A | 100 | 100 | 100 | 170 | 113 | 140 |
| W419B | 100 | 80 | 100 | 156 | 115 | 147 |
| W455A | 0 | 100 | 75 | NA | 166 | 146 |
| W460C | 40 | 40 | 60 | 148 | 138 | 156 |
| WEOS1 | 80 | 100 | 100 | 143 | 104 | 145 |

**Table 2.** Table of Tukey's HSD test values for the difference in Time to Scape Emergence across each of the three treatment groups. There is a highly significant difference in mean time to scape emergence between the 6-week and 0-week groups, as well as between the 6-week and 12-week groups. On average, bulbs from the 6-week group developed scapes approximately 28 days faster than bulbs from the 0-week group. There was not a significant difference in mean time to scape emergence between the 0-week and 12-week groups.

| Comparison of vernalization times | Difference in mean time to scape emergence (days) | *p*-Value | Significance |
|---|---|---|---|
| 6 weeks:0 weeks | −27.69 | 0.00 | *** |
| 12 weeks:0 weeks | −2.36 | 0.74 | NS |
| 12 weeks:6 weeks | 25.33 | 0.00 | *** |

*** indicates significance at α = 0.001 level; NS = not significant at α = 0.05 level.

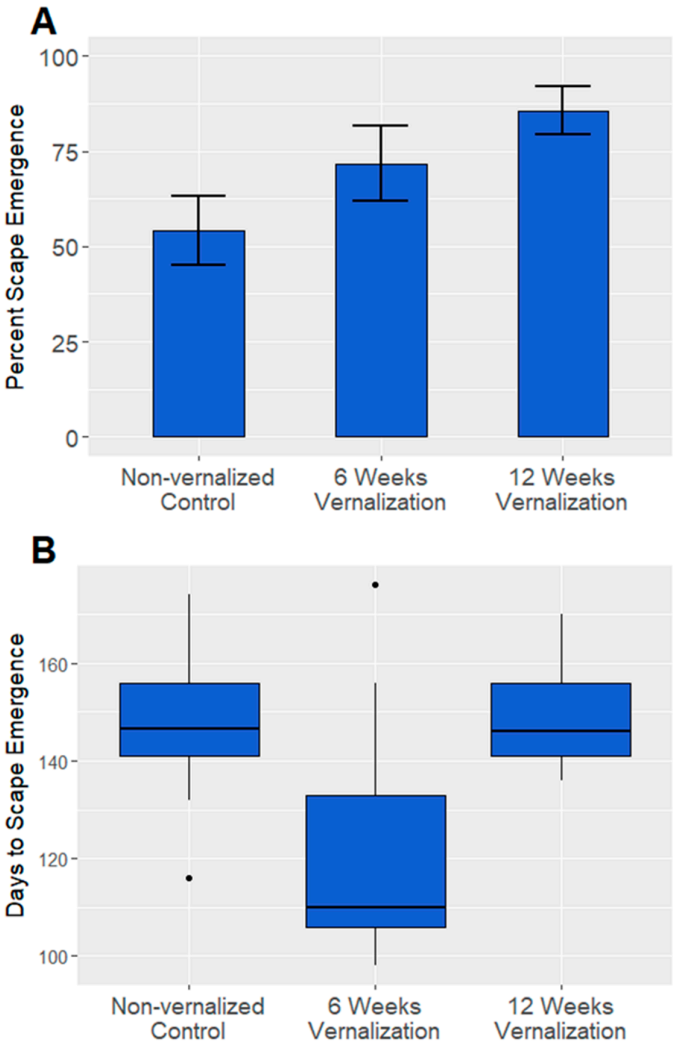


**Figure 2.** (**A**) Proportion of plants that underwent floral initiation under each vernalization treatment and the non-vernalized control group. (**B**) Days to scape emergence across each vernalization treatment group, aggregated data from each variety.

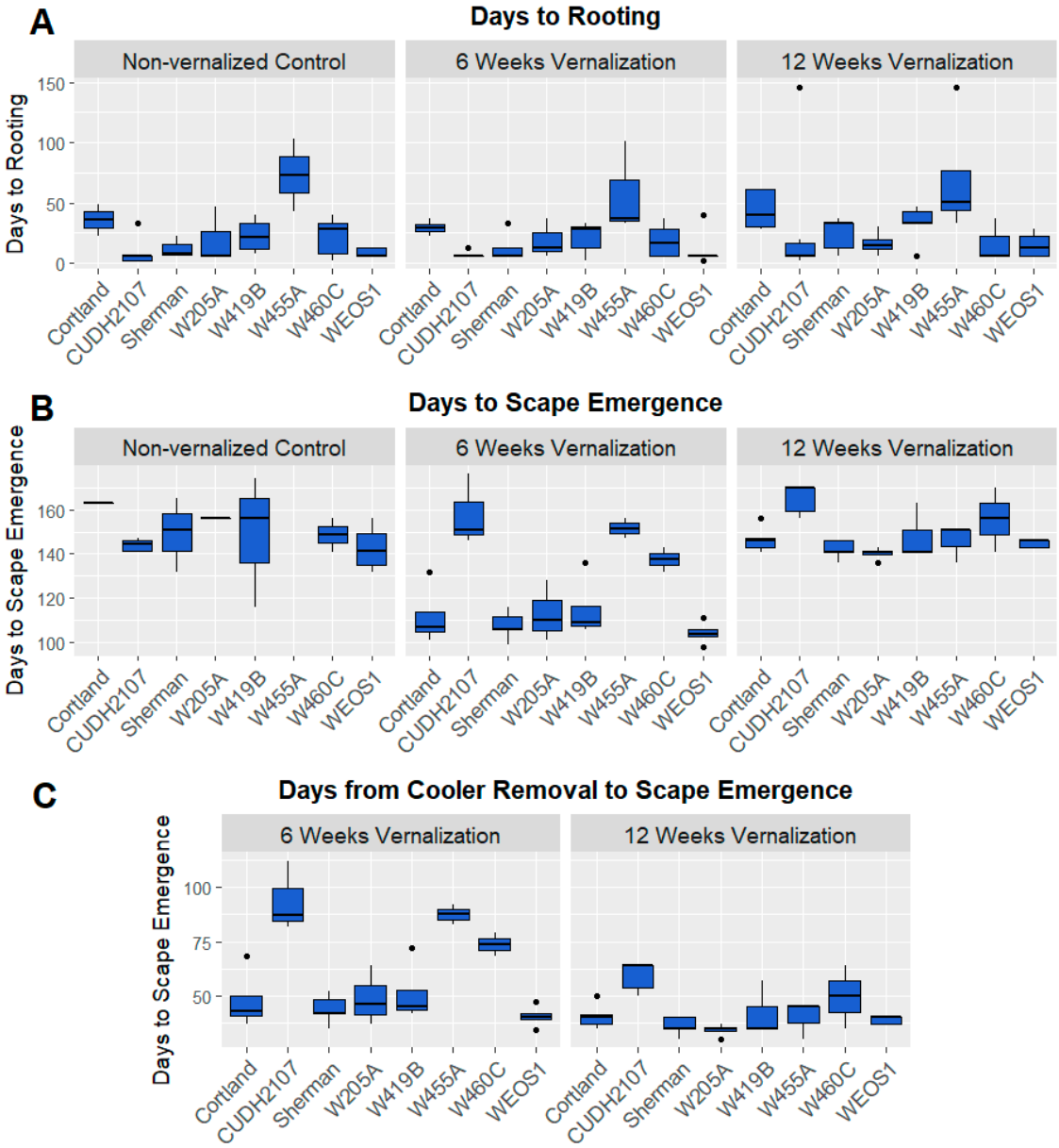


**Figure 3.** (**A**) Time to rooting among varieties and vernalization treatment groups following a 4-h treatment with 15% hydrogen peroxide. All bulbs were treated with hydrogen peroxide following harvest and curing, but before any vernalization treatments. The plants were monitored for root initiation through all stages of the experiment. (**B**) Days from planting to scape emergence in each of the eight varieties under various vernalization treatments. The control group remained in the 20 °C greenhouse for the entirety of the experiment. (**C**) Days from when plants were removed from the vernalization treatment until initial scape emergence.

The plants were monitored for growth and development regularly during the experiment, including throughout vernalization in the 10 °C growth chamber. While there was visible growth throughout vernalization, albeit at a slower rate than in the greenhouse, scape emergence was not observed until plants from their respective treatment group were moved back into the 20 °C greenhouse. Interestingly, the

6-week and 12-week treatment groups required approximately the same amount of time for scape emergence once removed from the growth chamber (Figure 3C).

We have previously demonstrated that treatments with 10–30% hydrogen peroxide solutions serve to promote uniform endodormancy release and root initiation in “Cortland” and “Sherman” bulbs [14]. In this study, CUDH2107, “Sherman”, and WEOS1 bulbs treated with a 15% hydrogen peroxide solution were effectively released from endodormancy as expected, with an average of 13.3 days to visible root growth. However, “Cortland”, W205A, W419B, W455A, and W460C had lengthy and varied rooting times, both within and among varieties, with an average time to rooting of 30.9 days (Figure 3A). For each variety, the time to root development was consistent between the non-vernalized control and two vernalization treatment groups. We observed sprouting prior to root development in approximately four percent of bulbs, one of which was the outlier in CUDH2107 from the 12-week treatment group (Figure 3A). This deviation in growth progression was not unique to any one variety but was rather noted in one to two bulbs of each “Sherman”, “Cortland”, CUDH2107, and W455A.

## DISCUSSION

This study was conducted to assess the utility of forced dormancy release combined with threshold vernalization in the development of an annual cycle breeding system for long day onion. The primary advantage of using an annualization method such as this over a seedling vernalization system is that this procedure includes an opportunity to evaluate bulb phenotypes at each generation. This annualization strategy, which we refer to as bulb annualization, capitalizes on the enhanced rate of vernalization in sprouted bulbs compared to unsprouted bulbs [9,10]. By forcing bulbs to break endodormancy through treatment with hydrogen peroxide, we were able to achieve uniform scape emergence across eight varieties while shortening the vernalization treatment necessary for similar performance in dry, unsprouted bulbs. The goals of this work were to determine if vernalization after a forced bulb endodormancy release using hydrogen peroxide would result in uniform flowering in less time than dry bulb vernalization and to establish a benchmark for the optimum vernalization time under this strategy.

Consistent with our observations concerning optimum vernalization time, we found that vernalization improves the uniformity of scape emergence and the proportion of plants that undergo floral initiation but is not a requirement for flowering [15]. Six weeks of vernalization resulted in uniform scape emergence in five of eight varieties, with over 66% of scapes emerging in under 120 days post-planting (Figure 3B). Plants from these varieties produced scapes 31% earlier following 6 weeks of vernalization than when vernalized for 12 weeks (Table 1). The remaining three varieties, CUDH2107, W455A, and W460C, required more time for

scapes to emerge. In the case of W455A, later scape emergence may have been a result of delayed dormancy release. On average, W455A required approximately 250% more time to break dormancy than other varieties. This delayed response may have affected the uniformity of the vernalization treatment as 29% of the bulbs did not break dormancy until after they were removed from the growth chamber.

Large differences in the time to rooting may be attributable to the reduced efficacy of hydrogen peroxide as a treatment for dormancy release in bulbs grown under high disease pressure. On August 10, 2017, a hail storm damaged our onion nursery which resulted in extensive losses both in the field and in storage. Following the storm, the bulbs were treated with a copper fungicide to manage the presence of disease, but a high percentage of bulbs used in this experiment showed signs of early stage soft rot during preparation for the hydrogen peroxide treatment. In most cases, we were able to undercut the infection prior to placing bulbs into the hydrogen peroxide solution, and only four plants were lost due to soft rot in the greenhouse. We believe that this practice in combination with the hydrogen peroxide treatment itself contributed to the greatly reduced presence of bulb rot in the greenhouse, as many other bulbs that were harvested from this field were lost in storage due to disease. While the treatment seems to have been effective at mitigating losses from disease pressure, the effect on root initiation was not as strong as in previous years. Uniformity in rooting time among varieties was poor relative to previous work and the performance of “Cortland” and “Sherman” was consistent with results reported by D’Angelo and Goldman [14] for the year 2015, which was also a year with high disease pressure. This observation supports an earlier observation that a 4-h hydrogen peroxide treatment may be less effective at breaking dormancy in bulbs that were subject to high disease pressure [14]. A 2 h, or less, treatment with 20% hydrogen peroxide may have produced better results under these circumstances. There was, however, consistent performance in rooting time across the three treatment groups.

In continuing with this work, we expect that bulb annualization will uncover a range of responses to vernalization due to the procedure’s relatively short chilling time. Under current practices there has been low selection pressure for uniform flowering following short vernalization times because in biennial breeding systems, dry, unsprouted bulbs often remain in cold storage for up to 7 months. The eight varieties used in this experiment are tolerant to bolting in the field and are representative of germplasm used in both research and bulb production in Wisconsin. Five of these varieties demonstrated uniform scape emergence with only 6 weeks of vernalization. This suggests that implementing vernalization periods longer than 6 weeks in a bulb annualization system is not essential for breeding varieties that are well-adapted to northern climates, such as Wisconsin, and that breeding under these conditions will not inherently select for bolting susceptibility. It is possible that uniform scape emergence

can still be achieved with shorter vernalization, but this requires further investigation and may come at the risk of reducing uniformity in the vernalization response, thus potentially placing a bottleneck on populations that are segregating in their response to vernalization.

As plants completed their vernalization treatments and were brought back into the greenhouse, we found that both the 4 and 12-week treatment groups required approximately 40–50 days for scapes to emerge (Figure 3C). The additional vernalization time did not meaningfully expedite scape emergence once the plants were removed from the cooler, which indicates that the primary advantage of longer vernalization in the context of bulb annualization is enhanced uniformity among varieties. These results also suggest that the additional 6 weeks of chilling received by the 12-week treatment group over the 6-week group improved floral initiation, but delayed growth and serves as a possible explanation for why there is not a significant improvement in scape emergence time compared to the non-vernalized bulbs (Table 2). Given these findings, the optimum vernalization time for bulb annualization is likely between 6 and 12 weeks of chilling at 10 °C. This would allow for earlier scape emergence than was found in the 12-week group, but greater uniformity among varieties than in the 6-week group. These recommendations may vary by geographic region and relatedness of the material in a given breeding program. Furthermore, the response to vernalization following dormancy break may vary from our findings in environments with lower disease pressure. Under low disease pressure, a 4-h treatment with 20% hydrogen peroxide should result in uniform root development in less than 2 weeks when grown in a 20 °C greenhouse under a 16-h daylength [14]. Given this scenario, we would expect greater uniformity in scape emergence when vernalized for 6 weeks than was observed in this experiment because rapid, synchronous dormancy release would allow all plants to be vernalized in a non-dormant state for the same period.

Transitioning to a new breeding system comes with its own set of challenges and may require implementation of new techniques or breeding objectives. One such objective might include incorporating dwarf scape phenotypes into breeding populations [19]. As onion plants flower in the greenhouse, the center of gravity is raised and the plants can be easily knocked over and damaged, which occasionally causes the scapes to break. We have also noticed that in some varieties the scapes will elongate to such a height where the florets are damaged by heat from the high-pressure sodium lighting. We found the dwarf scape phenotype to be well-suited to addressing these issues as the scape tends to be sturdy, easily managed, and compact, all of which facilitate the process of making crosses in a greenhouse.

Further experimentation on bulb annualization conditions could yield improvements to the process. We have recently initiated a trial with breeding material from the University of Wisconsin's onion breeding program to test this annualization process in a comprehensive manner.

Several segregating families derived from inbred lines that had been grown in 2015, crossed in 2016, and massed in 2017 are being tested utilizing this annualization system. Refining conditions during several key developmental phases could result in more efficient vernalization as well as expedite both flowering and seed set. One area worth investigating is the effects of light intensity and light quality from supplemental lighting. Higher light intensity during chilling has been shown to increase the rate of vernalization in actively growing plants but did not have the same effect on unsprouted bulbs [20,21]. The walk-in growth chamber used in this experiment utilized ceiling-mounted fluorescent lights which were partially shaded by shelving units, which resulted in relatively low light intensity during vernalization. Increasing the light intensity and duration during vernalization may improve the uniformity of flowering under shorter periods of vernalization [22].

Bulb annualization will likely not halve the time required to develop new varieties, but it has the potential to reduce the time from initial crossing to varietal release by several years. Although offering a bulb evaluation allows bulb phenotypic traits to be evaluated, a bulb-inclusive annualization system does not provide an opportunity to score for storage traits. Evaluation of post-harvest traits related to firmness, storability, sprout suppression, and skin appearance will still require a biennial breeding strategy. Late stage trials should still be conducted on a biennial cycle to ensure that requirements for bulb storability traits are met. Through this work we developed the framework for an annual cycle breeding system that offered the ability to phenotype bulb traits at each generation (Figure 4). We have been able to demonstrate a feasible framework for an annual cycle despite facing adverse conditions coming into the bulb annualization procedure. In addition, we have identified aspects of the onion life cycle that would benefit from further refinement to ensure rapid growth and development in an annualization system. Success in developing an annual cycle breeding procedure will be an important advance for breeders, horticulturists, and geneticists alike.

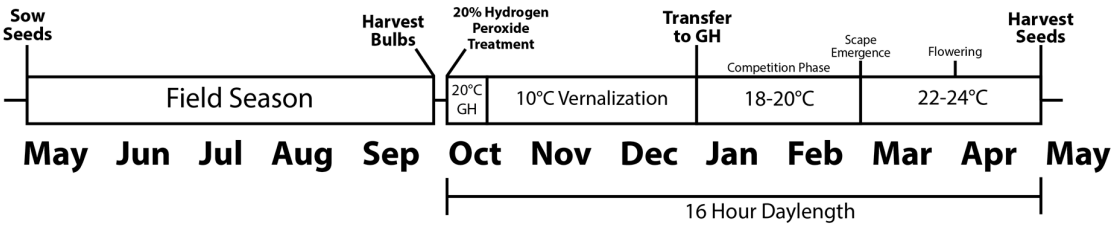


**Figure 4.** Proposed annualization timeline for long-day onion breeding.

## AUTHOR CONTRIBUTIONS

CD and IG designed the study. CD performed the experiments and analyzed the data. CD wrote the paper with input from IG.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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