Many Thanks for the 2005 Convention

John Migas — Saugatuck, Michigan

On behalf of the Lake Michigan Chapter I thank all the attendees to our recent ASA convention held in Holland, Michigan. Also, many thanks to the weatherman for the perfect week. On the last day of the convention, rain came steady and heavy. By the Monday after the convention, most flowers had fallen off. Talk about perfect timing.

Our volunteers deserve mentioning, starting with **Barb** and **Robert Wetzel,** for putting the "welcome bags" together; **Sharon** and **Dan Kunst,** for working the plant sale room; **Tad Dauksza,** for the plant list; **Rocky Voci,** for the program; **Sandra Wearne,** for a great job on the Web site; and many thanks to **Joe LaMantia** for registration.

Thank you to our garden stops: the staff of **Windmill Island** and **Veldheer Tulip Farm; the Alexander family;** the **Hartsuikers; Robert Hutchinson;** the **Hops; Karen** and **Herb Tews** of the Button Gallery; **Linda Charvat** and **Rosebay Nursery;** the **Willis family,** and the **Flower Basket.**

Many thanks, once again, to **Carol** and **Bruce Hop** along with the staff of **Wavecrest Nursery** for hosting lunch on Day 1 of the tours. Also thanks to **Joe LaMantia, Rocky Voci,** and my brother **Dan Migas** for preparing lunch on Day 2. I hope the choice of stops and the meals for lunch were satisfying. Thanks to **Brian Lernowich,** and **Tom** and **Chris Mallory** for following behind the bus to pick up any lost visitors. Thank you to **Dave Gregersen, Stacy Honson,** and **Dick Bont** for monitoring the par 3 contest, which was won by, you guessed it, **John Migas,** who practiced that shot for many weeks. Thanks also goes to **John Brown** who helped with the auction on Saturday night and who was our auctioneer.

Thanks to our speakers: **Carol Hop, Buddy Lee, Bill Pinkerton, Lee VerSchur,** and **Dr. Stan Hokanson.**

Thank you **Holiday Inn** and **Cardinal Bus Service.**

I thank **Buddy Lee** and **Bill Pinkerton** for helping out with stocking the plant sale room. Both Buddy and Bill arrived a few days early with the thought of sightseeing, rest, and relaxation; but, you guessed right again, I put them to work.

Once again, thanks to all who attended and all who volunteered for a very successful convention. Also, many thanks to the ASA board for giving our chapter the opportunity to host the 2005 convention. It was a lot of work, and an event I will always remember.

John Migas, *from Saugatuck in southwestern Michigan, was the driving force behind the Holland, Michigan, ASA convention. John has a harsh climate and short growing season to contend with when growing his 250 varieties of azaleas and 150 varieties of rhododendrons. He has been a member of the Society since 1996, but has been growing azaleas for 10 years and hybridizing them for three. He runs both a general contracting firm and a primarily wholesale nursery. Most of his sales have been to landscapers and retail nurserymen in the area, who are very surprised to find the broad range of azalea cultivars he is successfully growing in his five-acre display gardens. He has found membership in the Society to be very rewarding, and he hopes to increase interest in the ASA in his area, and to increase the activity of the Lake Michigan Chapter.*

Polyploidization of Evergreen Azaleas

Caitlin Klimavicz — Vienna, Virginia

Introduction

The characteristics that separate polyploid azaleas from diploid azaleas are the tendency of polyploids to have thick leaves and petals, dark green foliage, blight-resistant flowers, long-lasting flowers, increased opportunity to cross with deciduous azaleas, and a better marketability due to the fact that they look healthier year round (1). The purpose of this project, spanning over two years, was to determine a reliable and easily repeatable way to produce polyploid azaleas.

Diploid azaleas have 26 chromosomes (13 pairs). The greatest number of chromosomes ever recorded was 152 (76 pairs) (2). Some well known polyploids include: 'Haru-no-sono', 'Wako', 'Taihei', and 'Getsutoku'. There are two types of polyploid azaleas, allopolyploids and autopolyploids. Autopolyploids are usually sterile because of the odd number of chromosomes, while allopolyploids are usually fertile (3).

I have worked with my father for many years on a hybridization program aimed at developing sturdy azalea varieties that are better able to survive neglect and still look good. Specific hybridization objectives include a plant that is vigorous, disease- and insect-resistant, florifer-

ous, and has a flower that is long lasting. Polyploidization should help us reach many of our goals. Prior to my work on this project, our efforts to produce polyploid azaleas were limited to cross-pollination, and the results were relatively poor.

Azalea hybridizers sometimes wonder why a specific cross results in significantly lower germination rates when compared to other crosses. Early in this project, I designed an experiment to prove that when seeds have an abnormal number of chromosomes then germination is extremely limited. This phenomenon appeared to be directly related to whether one of the parent plants was a polyploid azalea.

In an attempt to find an easily repeatable method to produce polyploid azaleas with correspondingly higher germination rates, radiation was administered to the seeds before germination. Radiation is just one experimental technique to induce polyploidy. Many techniques have been tested, and the most successful to date has been treatment of the plant with colchicine chemicals. However, this technique is difficult in application and the results are often inconsistent (2).

Procedure

Seeds from known diploid and polyploid azaleas were collected and tested to determine if more diploid azalea seeds and X-rayed azalea seeds would germinate than polyploid azalea seeds. The seeds were then divided into three groups, each weighing 1.7 grams: two diploid groups and one polyploid group. One diploid group was X-rayed ten times (total of one second) at 15.0 mVs (millivolts per second). The X-ray machine, 123 kVp (kilovolts per second), was set 41 cm away from the envelope. Each group of seeds was planted in a separate container. The containers were placed under grow-lights and watered. After the seeds germinated, the number of seedlings in each container was counted.

Stomata measurements were taken to determine if the seeds that were X-rayed are polyploids. To measure the stomata of the azaleas, a microscope was calibrated using a micro ruler and the lines built into the eyepiece. Then leaves were collected from the plants to test. The leaves were labeled with a permanent marker on the topside of the leaves. The underside of the leaf was painted with clear nail polish. When the nail polish dried, it was peeled off the leaf. The clear piece of nail polish was placed on a clean slide with a drop of water. A cover slip was placed on top. Excess water was squeezed from under the cover slip, and the slide was labeled with a permanent marker. The slide was placed under the microscope and the microscope was focused on the leaf's stomata. The lines in the eyepiece that were calibrated earlier were used to measure the length and the width of three different stomata on each leaf.

Results

The number of seedlings in the diploid container that was not X-rayed was 264. The polyploid container had only one seed germinate. The X-rayed container had nine seeds that germinated. Seeds with an abnormal number of chromosomes do not germinate as readily as normal diploid seeds, therefore making polyploid azaleas more difficult to hybridize. The experiment determined that loss of germination was directly related to whether one of the parent plants was a polyploid azalea. There was also loss of germination in the X-rayed seeds, yet not to as great an extent (see Figure 1).

The next phase of the project was to determine if the X-rayed azaleas were actually polyploid. Stomata measurements can be used as a means for determining if an azalea is a polyploid. Based upon the two major groups of points, it appears that the X-rayed azaleas are polyploids. A statistical t-test was performed to determine if the two populations were statistically different. The test confirmed that they are different populations (see Figure 1).

Azaleas normally have two nuclear organizers. This picture clearly shows the four nuclear organizers present in the nucleus of an X-rayed azalea root tip cell (see Photo 1). This is also strong evidence that there are more chromosomes than normal in this cell, i.e., polyploidy (4).

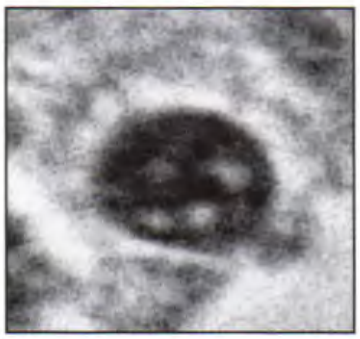


Photo 1. Nuclear organizers in the nucleus of an X-rayed azalea root tip cell.

Discussion

Using standard azalea crossing techniques as a means to produce polyploid azaleas does not produce acceptable levels of germination. As determined in the first phase of this project, the germination rates for a polyploid cross is significantly lower than normal diploid x diploid crosses. You can see in the graph of the number of plants that actually germinated, the number of diploid seeds that germinated was 264, but for the same mass of seed (measured on an electronic triple beam balance) only 1 polyploid crossed seed germinated and 10 of the X-rayed seeds germinated. That's a huge difference from 264 diploid crossed seeds (see bar graph, Figure 2).

If we want to induce polyploidy, we have to have some way to measure if we actually produced a polyploid azalea. The best way to measure polyploidy is to count the chromosomes under a microscope. This technique did not work in this case because it was too late in the year to get active root tips. The polyploidy in this case was taken into account by using stomata measurements. This is where the length and the width of stomata on each leaf are measured to

Figure 1. Comparison of Stomata Sizes

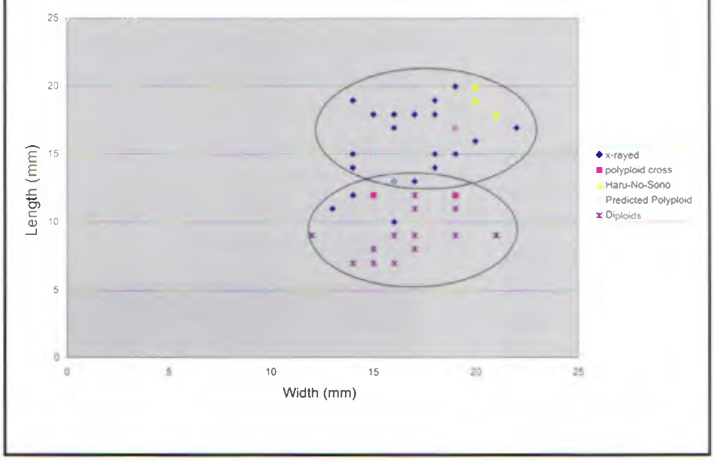
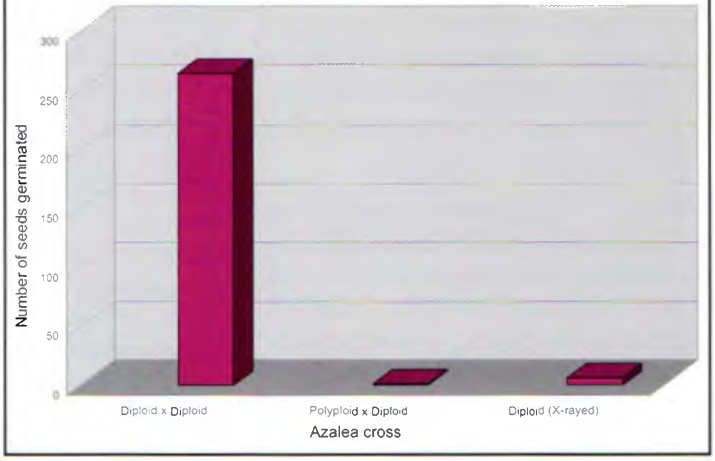


Figure 2. Germination of Different Azalea Crosses



determine the lengths-to-widths ratio that should vary between diploid and polyploid azaleas. Another technique that could have been used was FLOW (Flow spectrometer, particle analyzing system). FLOW is a system that measures the amount of genetic material in a sample and not how many chromosomes; therefore it is not very effective in determining polyploidy (4).

The cross-pollinated polyploid seeds were tested to determine if in fact there were embryos inside the seeds or if the lack of embryos was causing the lack of germination. To accomplish this test, seeds were soaked in Carmen dye for several minutes. If they floated, it was a sign that there might not be an embryo inside. Then, once the dye soaked in to the seeds, they could be looked at under a microscope to see the embryo. It was determined, using this method, that most of the seeds from the polyploid cross did contain embryos. This leads us to believe that the cause of decreased germination was not lack of embryos (4).

This project was not perfect, and there were two main places where error could have occurred. The first was in the measurements that were taken of the stomata. These measurements were done on a very small scale, so it would not have been hard to make an error. That is why three measurements were taken from every leaf. The second possible point of error is that even though the stomata measurements are designed to reveal the polyploidy of the azaleas, it is not the same as actually counting the chromosomes. Therefore, even if the measurements are performed correctly, it is not an absolute measure of polyploidy.

Conclusion

The main problem is that so far there is no reliable and easily repeatable way to generate polyploid azaleas. Right now there are three ways of producing possible polyploid azaleas: to cross-pollinate, to treat the actual plant with colchicines, or to expose the seeds to radiation (2). The hypothesis is that X-raying azalea seeds ia a more reliable and easier-to-repeat method for producing polyploid seedlings than cross-pollination.

Even though the data seems to suggest that radiation can produce polyploid azaleas, the question this project initially set out to answer definitely remains uncertain. The initial question was, "Is there a reliable and easily repeatable way to generate polyploid azaleas?" The answer given by the stomata measurements concludes that radiation is a reliable way to generate polyploid azaleas. Yet on the other hand, without an actual chromosome count one cannot be certain that the X-rayed seeds were actually polyploid. The germination rate for the X-rayed seeds was still very low compared to that for diploid seeds, and the radiation absorbed by the seeds could cause other problems down the line that have not been observed yet in this stage of growth. This reduced germination rate also impacts the ability of this method to easily generate new and exciting polyploid azaleas.

Future experiments could include actually counting the chromosomes, or testing the other methods, such as the chemical treatments applied when the plant is fully grown.

Acknowledgments

I would like to thank Dr. Robert Griesbach for his help with the measuring of the chromosome count and looking at the seeds for embryos. I would also like to thank him for his many ideas and suggestions that have improved my project. I would also like to thank Dr. John Klimavicz of Ashburn Farm Animal Hospital for the use of their X-ray machine.

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Caitlin Klimavicz *(shown in Photo 2) is currently a junior at James Madison High School in Vienna, Virginia. She has regularly attended azalea meetings with the Northern Virginia Chapter since 1989. She has won first place two years in a row at her high school science fair, and received a first and second place award in the regional science fair based on this project. Her first science project was "The Effect of Polyploidy on Azalea Seed Germination," which was the preliminary step in working to create better polyploids because the main problem with polyploids was their lack of germination in crosses. She hoped that irradiating the seed would not cut down on germination to the extent that the cross-pollinating did, but at the same time create the positive effects of polyploidy.*

Photo 2. Caitlin Klimavicz at work at the microscope.

Society News

Highlights of the Michigan Convention Meetings

Board of Directors Meetings

The board of directors met in Holland, Michigan, on May 19 and 22, 2005. Reports from the treasurer, azalea city committee, membership committee, the editor, the 2005 convention committee, the archives committee, and from representatives for future conventions, were accepted as presented with no major actions taken by the board. Joe Schild reported that McComb, Mississippi, has been accepted and designated as an "Azalea City." Current and previous articles on the various subjects in ***The Azalean*** cover the details of the reports. The board established the nominating committee for the 2006 elections. Buddy Lee, John Migas, and Carlton LeMond will serve on the nominating committee.

On other fronts, the board has authorized monies to develop a new color brochure. We have a rough draft of the new presentation that shows real promise. With Carol Segree in the lead, Bill McDavit, Barbara Stump, and Bob Hobbs will be working on a final draft for the board. It is hoped that the changes will help in attracting new members, the lifeblood of the Society.

The Society's Annual Meeting

At a previous meeting, the board of directors reviewed and approved an amendment to the by-laws to be submitted to the membership. The members present at the 2005 annual meeting voted to approve the change in wording. The revised Article IX. C now reads as follows:

> "All funds of the Society shall be deposited from time to time to the credit of the ASA in such bank, trust company, or other depository or depositories as the Executive Committee may select."

To support the intent of the revised by-laws, the board established an investment committee whose initial duties include the development of investment policies and reporting their recommendations to the board of directors.

In the elections for national office, Mary Rutley, Aaron Cook, and Dr. Joe Coleman were elected to two-year terms as directors. Bob Stelloh was re-elected treasurer and John Brown was re-elected as secretary.

Bill Miller was awarded the Best Article in ***The Azalean*** Award for 2004 for his article "Ben Morrison and His Azaleas," which appeared in the Fall 2004 issue.

Bob Stelloh was recognized for his many years of service to the Society. Fellow members chipped in on a state-of-the-art computer. However, Bob was riding in the automobile that would have carried his award, and to keep this a surprise, he received only the mouse at the convention. The mouse was mighty enough that Bob was rendered more or less speechless. Congratulations to Bob with appreciation for his service.

Respectfully submitted,
John Brown, Secretary