A Grower's Guide to Self and Cross-Incompatibility in Apple

Ben Orcheski and Susan Brown
Department of Horticulture,
Cornell University, NYSAES,
Geneva, NY

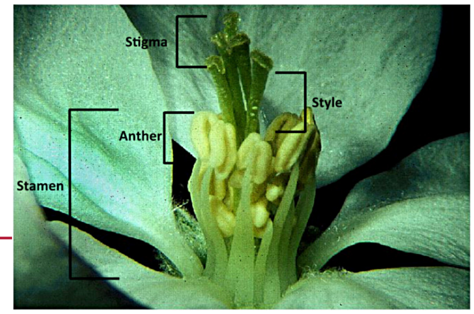

Figure 1. Parts of an apple flower (Photo courtesy of Martin Goffinet).

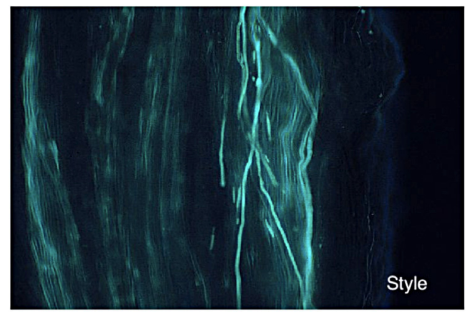

Figure 2. Pollen grains growing down the style of an apple flower (Photo courtesy of Martin Goffinet).

Pollination is a critical step in the production of quality apples. While many environmental factors can lead to poor pollination and lessen fruit set, the genetic makeup of the apple varieties in an orchard and their compatibility are crucial determinants of pollination success. The cultivated apple (*Malus x domestica*) along with many other economically important members of the *Rosaceae* or rose family (pears, peaches, plums, sweet and tart cherry) contain a complex biochemical system that prevents the variety's (also called a cultivar) own pollen from fertilizing the ovules, the initial step in fruit development (Hua et al., 2008). This system, known as self-incompatibility (SI) imposes many constraints upon both the apple grower and breeder, but in nature it has the benefit of keeping apple diverse. As a so-called "outcrosser", this ensures that future apple offspring would differ from their parents, giving them greater adaptive ability against diseases and stresses. We will describe how SI works, and what growers can do to overcome the challenges that SI can present. Our knowledge of self- and cross-incompatibility has evolved over the last few decades and new tools (molecular markers) are available to reduce the challenges posed by SI.

> **"Our knowledge of self- and cross-incompatibility has evolved over the last few decades and new tools (molecular markers) are available to reduce the challenges posed by self-incompatibility. When commercial growers grow one or more of the top 15 U.S. varieties, they are likely to encounter a situation where their trees are only partially compatible. Choosing fully compatible varieties out of the top 15 to plant together can be a daunting task. However, proper orchard planning can circumvent some of the problems of cross- incompatibility."**

Understanding Self-Incompatibility

What is Self-Incompatibility and How Does it Work? Before we delve into the workings of SI, it is essential to have an understanding of how fertilization is accomplished in an apple flower (Figure 1). In the orchard, successful pollination requires both a pollinizer and a pollinator. The pollinizer is the variety that produces the pollen, while the pollinator is the vector that transfers pollen between flowers. Bees are typically the most common pollinators of apple, as they readily consume both the nectar and pollen from the flowers as a food source. Bees are an essential component of a healthy orchard as apples are never wind pollinated (Way, 1995). When pollen produced in the roughly twenty anthers in an apple flower is shed, bees transfer pollen grains from one cultivar to one of the five stigmas on another cultivar. Pollen then germinates and begins to grow down through the style towards the ovule it will eventually fertilize (Figure 1). The ovule, or "small egg" will become a seed after successful fertilization by the pollen. On a pollen grain's journey through the style, one of two things will happen (Figure 2): 1) if it is a "compatible" pollen grain, it will continue to grow until it reaches the ovule; 2) if it is "incompatible" then the style will stop the pollen tube growth, ensuring it never reaches the ovule (Franklin-Tong and Franklin, 2003). Complete fertilization of an apple flower is extremely important for producing high quality fruit. If only some of the ovules are fertilized, the fruit will only grow in the areas where fertilization took place, so that uneven fertilization in the five seed cavities (carpels) results in small or misshapen fruit. The reason for this is that the developing seeds release hormones, which cause the ovary (fruit) to expand (Devoghalaere et al., 2012).

What Makes Pollen Compatible or Incompatible? The self-incompatibility system that operates in the flower determines whether the pollen grain will grow or die. As the pollen germinates and develops a pollen tube that grows down through the style, the style secretes molecules (substances) that interact with molecules in the pollen. Via this interaction, the style will recognize the pollen as either compatible (not from itself or a close relative) or incompatible (from itself or a closely related variety). The molecules released from the style will then stop the growth of the incompatible pollen, while the compatible pollen can grow and complete the fertilization process (Halász et al., 2006). Figure 2 shows pollen tube growth in the style.

Self-Incompatibility Nomenclature: The term used to describe the main component of the SI system is the S-allele, with 'S' referring to self-incompatibility. Each apple variety has either two or three S-alleles, depending upon whether they have two sets of chromosomes (diploid) or three (triploid). Each unique S-allele is assigned a number, and the two (or three) S-alleles of a variety are defined as its S-genotype (Broothaerts et al., 2004; Hegedûs, 2006; Long et al., 2010). For example, 'Golden Delicious' has both the S_2 and S_3 alleles, so its S-genotype would be written as S_2S_3. 'Jonagold', a triploid variety, has three alleles S_2, S_3 (from its 'Golden

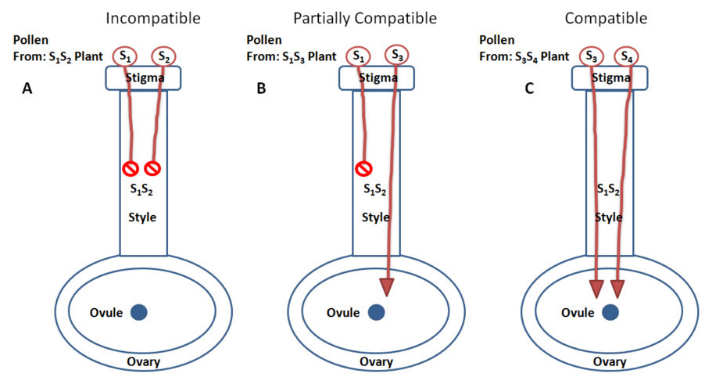

Figure 3. Diagrams of the three possible reactions in crossing two apple varieties.

Delicious' mother) and S_9 (from its 'Jonathan' father), so its S-genotype is $S_2S_3S_9$. While a cultivar such as 'Golden Delicious' has an S-genotype composed of two S-alleles, the pollen it produces will only have one of the S-alleles. Therefore, 'Golden Delicious' pollen is either S_2 or S_3.The partitioning of S-alleles in triploid varieties will be discussed later.

To date there have been about 28 S-alleles identified in the pool of apple varieties and species (apple germplasm), but the distribution is not even (Hegedûs, 2006). Many of these alleles are "proven" and well established, while some others will likely be revised or eliminated as methods for S-allele detection and verification become more accurate. This is why published reports have two different S-allele designations for some varieties, which needs to be resolved. More S-alleles will also be discovered in previously untested varieties, wild *Malus* species, crabapples, and new material from breeding programs.

Figure 3 presents the three possible situations that occur when pollen grains from various parents germinate on a stigma (Halász et al., 2006). In situation A, pollen grains from the same flower or variety with the same S-genotype germinate on the stigma. Pollen from this source is incompatible since both pollen grains match the S-alleles of the parent. After germination both the S_1 and S_2 pollen grains will stop growing. In situation B, pollen grains from an S_1S_3 flower land on the stigma and germinate. Pollen from this source is partially compatible because only the S_3 pollen will continue to grow down the style while the growth of the S_1 will be stopped. Finally, C represents a situation where a fully compatible pollen source pollinizes the flower. Since the S_3 and S_4 pollen grains do not have S-alleles in common with the variety, both grains will continue to germinate and depending on their rate of growth, one will fertilize the ovule. Although both are able to fertilize the ovule, some S-alleles have a competitive advantage in terms of growth rate over others.

How Do We Know the S-Genotype of an Apple Variety? The concept of SI has been known for well over a century but only in the past thirty years has the mechanism been sorted out (Halász et al., 2006). Before this time, a variety's S-genotype could never be definitively determined. Rather, the S-genotype was inferred through crosses to other varieties, which led to the concept of "incompatibility groups". Varieties that were fully or partially incompatible were in the same incompatibility group. For example, a researcher might perform a series of crosses with 'Macoun' and find that no fruit were produced when it was pollinated with 'Victory', some fruit set when 'McIntosh' pollen was used and plenty of fruit set when 'Fuji' pollen was applied. From these data the researcher could infer that 'Macoun', 'Victory' and 'McIntosh' were in the same incompatibility group while 'Fuji' was in a separate group. However, the researcher had no idea of which particular S-alleles made up each variety's S-genotype. The viability of the pollen and environmental conditions may also influence the outcome and result in potentially false conclusions. If the 'Fuji' pollen had been stored too long or if the weather was not conducive to pollen tube growth, fertilization could be negatively affected and a researcher might conclude that 'Fuji' was also incompatible. For this reason, pollination studies were conducted over several years to guard against faulty conclusions. While pollination tests continue to be used, they are being aided by modern genetic techniques.

Apple breeders are continually releasing new and improved varieties to the public. Since they are new to the market and growers have no previous experience with them, it is important to determine their S-genotype so growers can plan compatible combinations of varieties for new orchards. Today, S-alleles and incompatibility groups are much easier to determine with the help of molecular markers (Long et al., 2010). Molecular markers are differences in DNA or protein sequence that "tag" or "mark" a trait, in this case S-alleles. Since S-alleles are themselves DNA, and every S-allele is unique, each one will have its own molecular marker. Therefore, creating a molecular marker for an S-allele is done by finding the DNA or protein sequence that makes it different from all other known S-alleles. This can be done by isolating either the S-allele proteins or the genes coding for the S-allele proteins in a variety. The two different protein and gene sequences can either be distinguished by their size or from determining their sequence. Once a variety's S-genotype is known, its fertility with other varieties is easily explained. Let us now reconsider the 'Macoun' crosses in the above example. Using molecular markers the following S-genotypes have been elucidated: 'Macoun' (S_3S_{25}), 'Victory' (S_3S_{25}), 'McIntosh' ($S_{10}S_{25}$), and 'Fuji' (S_1S_9). The fertility of the crosses is now easily explained. 'Macoun' and 'Victory' are completely infertile since they share the same S-genotype. The 'Macoun' by 'McIntosh' cross was partially fertile because the two only share one S-allele in common. Finally, 'Macoun' by 'Fuji' is fully fertile because the two varieties don't share any S-alleles.

Cross-Incompatibility and Orchard Planning

S-Allele Diversity: The need for varieties that combine superior fruit quality as well as resistance to pests and environmental stresses has had a significant impact on the diversity of S-alleles in commonly grown varieties. The widespread use of some varieties in breeding programs, namely 'Delicious', 'Golden Delicious', and 'Jonathan' have caused the S_2, S_3, and S_9 alleles to become over-represented (Broothaerts et al., 2004; Hegedûs, 2006). This lack of S-allele diversity is evident in Table 1, which presents the top 15 (~90% of production) varieties grown in the U.S (information from www.usapple.org). A total of 32 S-alleles are possible from the 15 varieties, and although there are roughly 28 known S-alleles, only 12 alleles are found in these top 15. Furthermore, the three most frequent S-alleles, S_2, S_3 and S_9, account for 47% (15 of 32) of the S-alleles.

Impact on Growers: When commercial growers grow one or more of the top 15 U.S. varieties, they are likely to encounter a situation where their trees are only partially compatible. For instance, in an orchard planted exclusively with 'Braeburn' (S_9S_{24}) and 'Delicious' (S_9S_{28}), only pollen with the S_{24} and S_{28} pollen can participate in fertilization. This may result in lower yields as compared with an orchard planted with fully compatible varieties.

Table 1. S alleles of the top 15 commercially grown apple varieties in the US.

Variety	Parentage	S alleles	Literature Source
Braeburn	'Granny Smith' x 'Lady Hamilton'	S9S24	Dreesen 2010, Hegedûs 2006, Broothaerts 2004
Delicious	Chance Seedling	S9S28	Hegedûs 2006-
Mutsu (Crispin)	'Golden Delicious' × 'Indo Apple'	S2S3S20	Dreesen 2010, Hegedûs 2006, Broothaerts 2004
Empire	'McIntosh' x 'Delicious'	S10S28	Broothaerts 2004-
Fuji	'Delicious' × 'Ralls Janet'	S1S9	Dreesen 2010, Hegedûs 2006, Broothaerts 2004
Gala (Royal)	'Kidd's Orange Red' × 'Golden Delicious'	S2S5	Dreesen 2010, Hegedûs 2006, Broothaerts 2004, Li 2010
GingerGold	('Golden Delicious' × ' Albemarle Pippin') × Open	S3S28	Hegedûs 2006, Broothaerts 2004
Golden Delicious	Chance Seedling	S2S3	Dreesen 2010, Hegedûs 2006, Broothaerts 2004, Li 2010
Granny Smith	Chance Seedling	S3S23	Dreesen 2010, Hegedûs 2006, Broothaerts 2004
Honeycrisp	'Keepsake' × 'Unknown'	S2S24	Hegedûs 2006, Broothaerts 2004
Idared	'Jonathan' × 'Wagener'	S3S7	Broothaerts 2004-
Jonagold	'Golden Delicious' × 'Jonathan'	S2S3S9	Hegedûs 2006, Broothaerts 2004
Jonathan	Chance Seedling	S7S9	Li 2010, Hegedûs 2006, Broothaerts 2004
McIntosh	Chance Seedling	S10S25	Dreesen 2010, Hegedûs 2006, Broothaerts 2004
Rome Beauty	Chance Seedling	S20S24	Hegedûs 2006, Broothaerts 2004

Choosing fully compatible varieties out of the top 15 to plant together can be a daunting task. However, proper orchard planning can circumvent some of the problems of low S-allele diversity. For example, if an orchard is to be planted with 'Braeburn' and 'Delicious', it is a good idea to plant a fully compatible buffer variety in between, such as 'Gala' (S_2S_5) (Way, 1995). Using this layout, the 'Gala' in the center row can fertilize both 'Braeburn' and 'Delicious', who in turn fertilize 'Gala'. An additional complication to orchard planning is the need for overlapping bloom dates. Different varieties differ markedly in their bloom dates, meaning that even if two varieties are fully compatible based on their S-genotypes, they may not be suitable for cross-pollination if they do not flower at the same time (Way, 1995).

Triploid Varieties: Table 1, shows that two of the top fifteen varieties grown in the U.S., 'Mutsu' ('Crispin') and 'Jonagold', have three S-alleles due to receiving a full set of chromosomes (an unreduced gamete) instead of the usual half set from their maternal parent, 'Golden Delicious'. These varieties have three sets of chromosomes and are referred to as triploid. While triploid varieties are equal to other varieties in fruit set and are often superior in fruit size, they produce very poor quality pollen making them useless as pollinizers (Way, 1995). This adds another layer of complexity to orchard planning because an extra pollinizer is needed. For example, a grower wants to plant an orchard of 'Jonagold' ($S_2S_3S_9$) using 'Fuji' (S_1S_9) as a pollinizer. However, if the grower ever wishes to harvest 'Fuji' apples, a third variety is needed to provide pollen for 'Fuji', as 'Jonagold' does not have viable pollen and thus will not be a pollen source for 'Fuji'. Other triploid varieties include: 'Adam's Pearmain' ($S_1S_3S_{10}$), 'Bohnapfel' ($S_9S_{16}S_{17}$), 'Boskoop' ($S_2S_3S_5$), 'Citron d'Hiver' ($S_3S_5S_{12}$) 'Gravenstein' ($S_4S_{11}S_{31}$), 'Kanada Reinette' ($S_1S_2S_3$), 'Karmijn' ($S_5S_7S_9$), 'Ribston Pippin' ($S_1S_9S_{21}$), 'Spigold' ($S_1S_2S_3$), 'Stayman Winesap' ($S_1S_7S_{19}$) and 'Wintercitroenen' ($S_3S_5S_6$) (Broothaerts et al., 2004; Hegedûs, 2006; Long et al., 2010; Dreesen et al., 2010). Triploids occur naturally in apple breeding populations, but vary in the frequency of occurrence. Some seed parents like 'Golden Delicious' are more likely to produce unreduced gametes.

Varieties and Their Parents: When the parentage of a variety is known, that information can be used to plan which varieties to avoid when planting a new orchard. For example the well-known variety 'Fuji' (S_1S_9) is a hybrid of 'Delicious' (S_9S_{28}) by 'Ralls Janet' (S_1S_2) (Sakurai et al., 2000). Therefore, an orchard planted with only 'Fuji' and 'Delicious' will be only partially compatible and may not be such good idea. If this hypothetical orchard were to be planted, using additional pollinizers would be advisable.

While many of our commercially planted varieties are the products of breeding programs, and have well established parentage, some of our best known varieties are of unknown parentage. These varieties including 'Delicious', 'Golden Delicious', 'Granny Smith' and 'McIntosh', are chance seedlings, and at best their parentage can only be inferred from their S-alleles (Ferree and Warrington, 2003). This is one reason why knowledge of S-alleles is so important for growers when planning an orchard, and also for breeders when making crosses to develop new varieties.

To demonstrate how S-alleles can be used to infer parentage of a variety, let's look at an example. 'Empire' was selected from a cross of 'Delicious' (S9S28) and McIntosh (S10S25). We can use a Punnett square (Figure 4) to determine the likely S-genotype of 'Empire'. The Punnett square shows the four possible S-genotypes that can be made from this cross, one of which is 'Empire'. Molecular markers can then be used to infer which S-genotype of 'Empire' is correct (S10S28). S-alleles have also indicated where the presumed parentage of a variety is wrong."

Sports, Parents and Pollen: Sports are naturally occurring mutations found on limbs of a parent variety. Buds from the mutant sport are propagated on rootstocks and made available to growers. Many sports of a variety can exist, and major commercial varieties can have dozens of named and patented sports. For example, 'Delicious' which has been around well over a hundred years has nearly fifty named sports (Nybom, 1990; www.freepatentsonline.com). While each sport has developed a unique mutation that makes it desirable to propagate, sports are essentially identical to their parent variety. For this reason sports have the same S-genotype as their parents and they should not be

		'Delicious' (S_9S_{28})	
		S_9	S_{28}
'McIntosh' ($S_{10}S_{25}$)	S_{10}	S_9S_{10}	$S_{10}S_{28}$ 'Empire'
	S_{25}	S_9S_{25}	$S_{25}S_{28}$

Figure 4. A Punnet square showing possible S-alleles of 'Empire' apple based on its parentage. Molecular markers determined the S-genotype to be $S_{10}S_{28}$.

used to pollinize one another or the variety from which they originated.

Crabapples as Pollen Sources: Another source of pollen that growers can take advantage of is the crabapple. Crabapple species can make excellent pollinizers for a number of reasons. They produce an abundance of flowers, and the large number of existing species and varieties ensures that a compatible bloom time can be found between the cultivar and the crabapple (Way, 1995). In addition, despite the use of some crabapple species in breeding programs, crabapple S-alleles appear to remain rare among cultivated apple, making them less likely to have compatibility issues with commercial apple varieties. For example, some varieties of the crabapple species *M. floribunda* contain the rare S_{16} allele. These varieties are also used in breeding programs as a source of scab resistance, yet the S_{16} allele remains extremely rare in cultivated apple (Dreesen et al., 2010). However, the continued use of crabapples in breeding could cause their S-alleles to become more prevalent in new varieties.

The obvious disadvantage of using crabapples as pollen sources is that they take up space that could be used for planting commercial varieties. Space can be conserved by top grafting crabapple to the cultivar, although the labor cost may be prohibitively expensive. Another problem with crabapples is that like many cultivars they can be biennial bearers, which would require two different crab varieties to be planted in an orchard. One final issue with the use of crabapples as pollen is that the species of crabapple may not be able to fertilize the apple cultivar. This is not an issue of S-alleles but rather failure of the seed to develop after fertilization with crabapple pollen (Way, 1995).

It is important to note that using crabapples as pollinizers will not affect the fruit quality of cultivars. The fruit characteristics are a product of the variety and are not affected by the pollen source. As long as crabapple pollen has fertilized all the ovules, the fruit should develop the same as if it were fertilized by another cultivar.

Conclusions

Self-incompatibility (SI) in apple is a simple, yet elegant system that prevents an apple from being fertilized by its own pollen or by varieties with similar pollen. While the biology and physiology of this phenomenon is fascinating, there is also a practical aspect to understanding SI. In breeding, difficulty arises from not being able to make crosses between two incompatible varieties. For growers, SI can make orchard planning difficult by preventing the planting of solid blocks of one variety or incompatible varieties in the same orchard. A grower with knowledge of cross-incompatibility has the power to properly choose the varieties planted in an orchard and in turn, maximize yield and quality.

Although the phenomenon has been known for over a century, the underlying principles of SI have only recently been elucidated. Research is rapidly progressing in many labs to better understand the SI system. One of the most important goals of this research is to improve the detection of S-alleles in current varieties and to uncover novel or rare S-alleles in the thousands of named varieties that currently exist. Improved detection will undoubtedly aide both the breeders who develop new varieties and the growers who cultivate them.

Despite the many challenges it poses, the self-incompatibility system has been a boon to apple, keeping it genetically diverse, despite intensive breeding efforts. This in turn allows the apple to evolve, adapt to changing environments, and ultimately thrive as both a species and a source of food and beauty for humans. SI has been critical to the evolution, domestication and continued use of the apple throughout the world. This is evidenced by the thousands of varieties currently available to both breeders and growers and the many more improved varieties that will be available in the future.

Literature Cited

Broothaerts, W., Nerum, I.V. and Keulemans, J. (2004) Update on and review of incompatibility (S-) genotypes of apple cultivars. *HortScience* 39(5): 943-947.

Devoghalaere, F., Doucen, T., Guitton, B., Keeling, J., Payne, W., Ling, T.J., Ross, J.J., Hallett, I.C., Gunaseelan, K., Dayatilake, G., Diak, R., Breen, K.C., Tustin, D.S., Costes, E., Chagne, D., Schaffer, R.J. and David, K.M. (2012) A genomics approach to understanding the role of auxin in apple (*Malus* x *domestica*) fruit size control. *BMC Plant Biolog*y 12:7.

Dreesen, R., Vanholme, B., Luyten, K., Wynsberghe, L., Fazio, G., Roldan-Ruiz, I., and Keulemans, J. (2010) Analysis of *Malus* S-RNase gene diversity based on a comparative study of old and modern apple cultivars and European wild apple. *Molecular Breeding* 26:693–709.

Ferree, D.C. and Warrington, I.J. (2003) Apples: botany, production, and uses. New York, NY: Publisher: CABI Pub.

Fields, Z.H. and Kaoa, A.T. (2008) Biochemical models for S-RNase-based self-incompatibility. *Molecular Plant* 1(4): 575–585.

Franklin-Tong, V.E. and Franklin, F.C.H. (2003) The different mechanisms of gametophytic self-incompatibility. *Phil. Trans. R. Soc. Lond.* 358: 1025–1032.

Halász, J., Hegedûs, A. and Pedryc, A. (2006) Review of the molecular background of self-incompatibility in rosaceous fruit trees. *International J. Hort. Sci.* 12(2): 7–18.

Hegedûs, A. (2006) Review of the self-incompatibility in apple (*Malus ×domestica* Borkh., syn. *Malus pumila* Mill.). *International J. Hort. Sci.*12(2): 31–36.

Hua, Z-H., Fields, A. and Kao, T.-H. (2008) Biochemical models for S-RNase-based self-incompatibility. *Molecular Plant* 1(4):575-585.

Long, S., Li, M., Han, H., Wang, K. and Li, T. (2010) Characterization of three new S-alleles and development of an S-allele-specific PCR system for rapidly identifying the S-genotype in apple cultivars. *Tree Genetics & Genomes* 6:161–168.

Nybom, H. (1990) DNA fingerprints in sports of 'Red Delicious' apples. *HortScience* 25(12): 1641-1642.

Sakurai, K., Brown, S.K. and Weeden, N. (2000) Self-incompatibility alleles of apple cultivars and advanced selections. *HortScience* 35(1):116–119.

Way, R. (1995) Pollination and fruit set of fruit crops. *Cornell Cooperative Extension Information Bulletin 237.*

www.freepatentsonline.com

www.usapple.org

Ben Orcheski is a PhD student working with Susan Brown. **Susan Brown** is a professor of Horticulture, located at Cornell University, Geneva Experiment Station who leads Cornell's apple breeding program. Ben Orcheski's research project involves studies of genes in inter-specific hybrids in apple. This article was part of Ben's extension assistantship responsibilities.