Current Red Clover Breeding Research

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Abstract. Red clover remains a globally important forage legume grown mostly in humid temperate climates on approximately 4 million ha. In the past 25 years global red clover seed production has stabilized around 10 million kg yr^{-1}, although there is evidence that seed production is increasing globally at around 0.34 million kg yr^{-1} since the early 2000s. Globally many red clover programs exist targeting specific global regions with a focus on improving persistence, biomass yield, seed yield, and a few other minor traits. Breeding programs are increasing persistence by selecting for specific disease resistances, specific agricultural conditions such as mixtures with grass, or specific climate or climate induced conditions such as winter survival or drought. A few programs are targeting biomass yield *per se*, however, these efforts are yielding slower selection progress and are often confounded with selection gains for improved persistence. Red clover seed yield improvement is another major breeding target. 1944 to 2017 Oregon U.S. seed production data indicates diploid red clover seed yields are improve at a rate of 7.1% per year. It is, however, unknown what proportion of this increase is due to improved genetics versus improved management. Furthermore, tetraploid red clover which has lower seed yields remains an area of continued breeding interest particularly in Europe. In addition to the major three breeding traits of persistence, biomass yield, and seed yield a few red clover breeding programs are pursuing other traits such as breeding for improved forage quality. Global red clover breeding efforts tend to be isolated from one another with little exchange of elite germplasm between global regions. Programs are often exploring local landraces or local wild/feral germplasm to expand their elite breeding pools.

Introduction
Red clover (*Trifolium pratense* L.) is a major forage legume grown in temperate climates around the world. Since the mid-1990s red clover seed production has stabilized around 10 million kg of seed produced per year (Riday, 2010). Assuming 2.75 kg ha^{-1} yr^{-1} of red clover seed usage this translates to an estimated 4 million global ha of red clover. Major seed producing countries in 2020 include: U.S.A. (5.00 million kg) (Anderson, 2014-2021), France (1.46 million kg), Canada (1.18 million kg [red clover usage data]) (Statistic Canada, 2022), Lithuania (0.81 million kg), Germany (0.71 million kg), Czechia (0.71 million kg), Sweden (0.54 million kg), Italy (0.48 million kg), and Denmark (0.43 million kg) (ESCAA, 2022). In Europe from 1997 to 2020 red clover production has been increasing at a rate of 0.09 million kg yr^{-1} (2.4% yr^{-1}; $P = 0.014$) (ESCAA, 2022). In the U.S. from 2003 to 2021 red clover seed production has been increasing at a rate of 0.10 million kg yr^{-1} (2.5% yr^{-1}; $P = 0.061$) (Young, 2005-2013; Anderson, 2014-2021). Europe and U.S. combined red clover seed production from 2003 to 2020 increase at a rate of 0.34 million kg yr-^{1} (4.8% yr^{-1}; $P < 0.0001$).
Red clover is utilized in intensive and extensive systems such as grown in monoculture or as a mixture, as a green manure, harvested as hay, silage, and/or baleage. Red clover is also grown in pasture systems to be grazed. Red clover is utilized as a winter annual in subtropical environments, as a short lived perennial (1 to 2 years) in many temperate systems, and as a perennial (3 to 4 years) in other temperate agricultural systems (Riday, 2010). Due to red clover's expected usage in a wide range of agricultural systems and climates, breeding targets are varied. However, red clover persistence remains the major trait being bred for. Closely tied to persistence is breeding for biomass yield with these two traits often conflated in red clover. Finally continued effort to increasing red clover seed yield remains.

Persistence
Historically, breeding for disease resistance has been one method of increasing red clover persistence. In North America current limited breeding for resistance to powdery mildew (*Erysiphe polygoni* D.C.), black patch (*Rhizoctonia leguminicola* Gough and Elliott), northern anthracnose (*Kabatiella caulivora* [Kirchn.] Karak.) and southern anthracnose (*Colletotrichum trifolii* Bain et Essary) is occurring. In Europe more intensive breeding for resistance to Sclerotinia crown rot (*Sclerotinia trifolium* Erikks.) (Frey et al., 2022) and southern anthracnose (Hartmann et al., 2022) is being pursued. European, N. American, S. American, and Australasia breeding programs are specifically breeding for red clover persistence when grown in mixture with forage grasses or at least targeting their cultivars for use in mixtures (Ford and Barrett, 2011; Hoekstra et al., 2018; Conaghan, 2018; Sindic and Riday, 2020, and Ergon and Bakken, 2022). A few groups are focusing on identifying red clover morphologies related to persistence with the hope of utilizing this information to

improve persistence (Inostroza et al., 2020). Every current red clover program is focusing on increasing red clover persistence for specific target climates including: northern latitude temperate cold climates; continental temperate mid-latitude climates; oceanic temperate mid-latitude climates, and humid sub-tropical climates (Dall'Agnol et al., 2021).

Biomass Yield

Closely related to persistence breeding is breeding for biomass yield. Reported biomass yield selection gains range from 0.17% to 1.39% per year (Riday, 2010; Grieder et al., 2019). All reported studies show that biomass yield gains through breeding are greater in later year stands compared to earlier year stands. This observation indicates that increased persistence is a key factor in biomass yield improvement. Furthermore percent selection progress for persistence has outpaced the percent selection gain percentages for biomass yield. Selection gains for biomass yield have been greater in tetraploid varieties compared to diploid varieties (Grieder et al., 2019). Selection for biomass yield is difficult. In the U.S. Dairy Forage Research Center red clover breeding program from 2004 to 2020 estimated narrow sense heritability on an individual plant basis based on a single vigor observation taken on four year old plants was estimated at $h^2 = 0.022 \pm 0.022$. This heritability estimated is based off of over 28,000 parent-offspring space planted phenotypic observation pairs. If repeated observation of the same plant over a four year period are taken or one observation of the same plant after each regrowth period (approximately 15 observations over the life of that plant) heritability on an individual plant basis increases to $h^2 = 0.153 \pm 0.022$. Estimated narrow sense heritability on a halfsib mean based on 40 individual plants per halfsib-family observed repeatedly was $h^2 = 0.644$. If remnant seed of superior selected halfsib families were used in selection schemes the effective heritability for selection gain purposes would be $eff.\_h^2 = 0.161$. These heritability observations indicate that selecting good looking surviving plants out four year old space planted red clover nurseries will lead to slower selection gains ($h = 0.148$) compared to selecting surviving plants out of red clover nurseries based on their average observed performance over that four year period ($h = 0.391$). While selecting random individuals from remnant seed of the best halfsib families based off of four years of observations on those halfsib families individual space plant progeny will have marginally even greater selection gains ($eff.\_h = 0.401$). If both parents are controlled during selection 1) by doing a paternity test on remnant seed and selecting for superior pollen-parents based on this test or 2) by maintaining parent plant nurseries and polycrossing superior parent plants after progeny testing parent plants, then selection gain would be doubled (i.e., $eff.\_h = 0.802$).

Seed Yield

Increasing red clover seed yields can decrease seed costs paid by producers, thereby increasing the attractiveness of utilizing this forage legume. This has led to numerous studies examining red clover plant traits associated with higher seed yield (Vleugels et al., 2019; Jing et al., 2021). In particular trying to determine the basis and overcome lower seed yield in tetraploid red clover is an ongoing process as tetraploid seed yields remain 20% to 50% below diploid seed yields (Jing et al., 2021). European researchers have concluded that less selection for seed yield has happened and furthermore, observed that seed yield heritability is low (Vleugels et al., 2019). In the U.S., however, a steady increase in seed yield per hectare has been observed. In the U.S. in 2017, 78.6% of red clover seed production hectarage was located in the state of Oregon (USDA-NASS, 1944-2017). Since 1944 Oregon seed yield ha^{-1} has increased from 167 kg ha^{-1} to 656 kg ha^{-1} in 2017 with a linear model indicating a 7.6 kg ha^{-1} seed yield increase per year or a 7.1% gain per year (seed yield = 7.64 x year – 14745; $P < 0.0001$ $r^2 = 0.892$). It is unclear what proportion of the increase is due to selection gain versus improved seed production management. Clearly improved management has played a role as seed production specialization and seed industry consolidation have reduced the number of red clover seed producing farms in Oregon from 824 farms in 1944 (representing 0.6% of U.S. red clover seed producing farms) with an average 5.7 ha $farm^{-1}$ of red clover seed production to 182 farms in 2017 (representing 60.7% of U.S. red clover seed producing farms) with an average of 38.3 ha $farm^{-1}$ red clover seed production. One notable observation is that seed yields in the U.S. excluding Oregon have not increased as dramatically (1944 at 56 kg ha^{-1} to 2017 at 308 kg ha^{-1}). Furthermore in 2017 in the U.S. excluding Oregon the average number of red clover seed production ha on a farm were much smaller at 16.1 ha when compared to Oregon, suggesting that red clover seed production activity outside of Oregon in the U.S. may be less management intensive resulting in lower seed yields. It is unclear how significant variety by production climate interactions (GE) are. For biomass yield GE is large, particularly as red clover is grown all the way from sub-tropical to cold continental climates. As with forage yield, red clover seed production climates vary tremendously across the globe.

Other Traits

In addition to persistence, biomass yield, and seed yield, small efforts have been devoted to breeding speciality traits such as 2,4-D resistant red clover (Quesenberry et al., 2015; Riday, 2016), red clover with varying phytoestrogen content (Little et al., 2017), and attempts to improve forage quality (Tucak et al., 2021). Work in this area has led to some released material, however, compared to other major traits efforts in these areas have been minor.

Conclusions and Implications

Due to red clover's global utilization in many different types of agricultural situations, its hectarage being on a smaller scale than major cash commodity crops, and the red clover plant material itself not being a major cash crop, has led to the situation of globally lower profile red clover breeding efforts. Compared to commodity crops, clover breeding programs tend to be smaller and focus on targeting major traits and out of necessity attempting to create varieties with broader adaptation. These efforts in turn likely have led to lower selection gains relative to major inbred and hybrid commodity species. Many breeding programs are still exploring climate local red clover landraces and wild accessions for potential use in elite red clover breeding pools (Jones et al., 2018; Petrauskas et al., 2020; Dall'Agnol et al., 2021; Zanotto et al., 2021). This is indicative of a fragmented elite red clover breeding landscape were elite global red clover breeding programs find less utility or value in incorporating elite red clover germplasm from other global sources. This fragmentation is likely exacerbated by red clover's primary breeding challenge being persistence. Each climate region therefore has unique persistence challenges that make global exchanges of elite germplasm less effective. Globally red clover breeding could use more communication and exchange among disparate often isolated small red clover breeding programs. Red clover breeding programs could also utilize more sophisticated phenotyping and genotyping technologies. Resource constraints are the major limitation to incorporating new breeding technologies. Although many newer breeding aids are becoming cost effective for red clover breeding programs (Riday, 2013; Sindic and Riday, 2020; Kronenberg et al., 2021).

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