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PROLIFERATION AND DETECTION OF CONTAMINATION IN TURFGRASS VEGETATIVE PROPAGATION

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ABSTRACT

Turfgrass cultivars are often vegetatively propagated clones which can be contaminated by genetic off-types of the same or closely related species. If a contaminant clone is more competitive than the intended cultivar there is potential for proliferation (increased contamination) during propagation. The objectives of this study were to estimate the rate of proliferation of a competitive genetic contaminant into a less competitive cultivar, and to estimate the likelihood of detecting a contaminant, once it occurs. The logistic equation was used to model the sigmoidal growth in cover, as a function of days since planting, for a weedy bermudagrass, *Cynodon dactylon* (L.) Pers 'PI-291586', grown without competition. The observed growth rate was depreciated in steps to simulate the growth of a hypothetically less competitive cultivar growing together with a small admixture of the more competitive clone. This was repeated in steps across a range of admixture rates. It was shown that a small rate of admixture, 0.001, of a contaminant with a 50% faster growth rate, could in one planting cycle proliferate 140 times in the planting. The detection of contamination through random sampling depends on the power required (the likelihood of finding a contaminant), the frequency of occurrence of the off-type, and the accuracy of diagnosis. While the accuracy of diagnosing contaminant grasses may vary, the binomial expectations of random sampling can be precisely estimated. The required number of samples is ln(1-power)/ln(purity), where the power is the likelihood of finding a contaminant and the purity is the predominance of the intended cultivar. The likelihood of detecting a genetic variation through random sampling is rare, unless many samples are analyzed. To be 95% sure of detecting a 0.8% contaminant, one would need to collect and analyze 373 samples, which is probably impractical. Therefore, visual sampling or other survey approaches are necessary in any quality assurance program.

Keywords: bermudagrass, breeding, competition, clone, cultivar, sod, sprig, *Cynodon dactylon*.

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INTRODUCTION

Turfgrass cultivars are often grown as individual clones. 'Tifdwarf', 'Tifway', and other hybrid bermudagrasses, *Cynodon* spp. used on golf courses and sports fields are sterile triploids ($2n = 27$) (Taliaferro et al., 1997). Propagated vegetatively, their genetic homogeneity can provide predictable turf performance, e.g., uniform play, appearance, and tolerance to wear and herbicides. Genetic off-types sometimes appear as patches in vegetatively propagated turf plantings, resulting in unacceptable turf characteristics. Off-types might arise in largely sterile clones by spontaneous somatic mutation (Caetano-Anolles, 1999), seedling infestation (Anderson et al., 2001), or admixture of other cultivars. Other warm-season turfgrasses, e.g., 'Floratam' St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, are also propagated vegetatively, and are thus also liable to disfiguration and loss of turfgrass quality by genetic off-types.

Whatever their method of initial occurrence, if genetic variants are undetected they can be perpetuated during turf production, distribution, and maintenance. Over time, more aggressive genetic variants should progressively increase in canopy area and biomass, compared with the intended cultivar. Cultivars are propagated through successive cycles of expansion from the original breeder's stock, through foundation stock and certified stock, which is sold to end users, thus there are staged opportunities during cultivar expansion for a contaminant to proliferate.

Vegetative biomass growth of eight genotypes of turfgrasses in five genera can be interpreted as exponential growth (Busey and Myers, 1979) with different base rates of exponential increase, depending on genotype, soil, growth period, and temperature. Although soil medium affected growth and there were some interactions with genotype, different genotypes nevertheless maintained nearly identical rank order with respect to growth rate in different soil media. Longer term experiments in the same study, including studies in both the greenhouse and in the field, also demonstrated exponential growth of turfgrasses for both biomass and canopy cover. A relatively slow growing turfgrass, bahiagrass (*Paspalum notatum* Flügge) maintained exponential growth through 103 days after planting. Various factors including irrigation, fertilization, and solar radiation could affect turfgrass growth but in the simplest approximation under favorable environmental conditions for different turfgrasses growth is exponential.

Because turfgrass growth is exponential it can be linearized for the early stages of field expansion by logarithmic transformation (Busey and Myers, 1979). But since canopy cover cannot exceed 100%, a confined propagation system has an imposed asymptote. For example, self-inhibition occurs for St. Augustinegrass sometime after 45% canopy cover is achieved. The vegetative growth of a spreading turfgrass thus conforms to a sigmoid or S-shaped distribution which is based on a simple model of exponential growth with an asymptote. The Gompertz model is an asymmetric, sigmoid curve which is useful in solving growth and similar problems such as seed germination (Tipton, 1984) and turfgrass evapotranspiration under progressive stress (Fernandez and Love, 1993), as well as turfgrass expansion (Busey and Myers, 1979). The logistic equation, although with limitations (Damgaard et al., 2002), provides a simpler and more adaptable method than

the Gompertz model for sigmoidal growth of individuals, populations, and species under competition (Weiner and Thomas, 1986). The logistic equation is based on exponential growth initially but includes a crowding term as resources are depleted and the population reaches carrying capacity or maximum surface area in the case of a spreading turfgrass and it includes a term pertaining to the symmetry of the curve.

Whereas the exponential growth of turfgrasses has been demonstrated as a general principle that can be applied to both slow-growing and fast-growing genotypes (Busey and Myers, 1979) and the logistic equation adds simple adjustments, maximum surface area which would be the same for all turfgrass populations, the use of the logistic equation might provide a useful model for competition among turfgrasses including contaminants.

Another quantitative problem of maintaining pure clonal plantings is the detection of admixture, once it occurs. Various methods of identification have been used for clonal turfgrass cultivars, e.g., morphology in St. Augustinegrass (Busey, 1986) and DNA amplification profiling in bermudagrass (Cataeno-Anolles, et al., 1995). To determine whether a population (e.g., a field of bermudagrass) is genetically homogeneous, samples are sometimes removed for diagnosis. The ability to detect an off-type is dependent on the type I and type II errors associated with a particular test, but also on the binomial expectations related to the number of samples. The latter can be estimated. The study had two objectives, to estimate the rate of proliferation of an aggressive genetic contaminant into a less aggressive cultivar, and to estimate the likelihood of detecting a contaminant, once it occurs.

MATERIALS AND METHODS

Growth of a single aggressive bermudagrass. A rapidly spreading bermudagrass, *Cynodon dactylon* PI-291586, was planted as sprigs from trays on 2 April 1983. The original canopy area, 3.82 m^2 covered trays, was planted in 1450 m^2 (0.00263 planting rate, area:area). Soil was Margate fine sand (hyperthermic, Mollic Psammaquent), 94% sand with pH 7.3, 13 mg P kg^{-1}, 41 mg K kg^{-1}, and 5.5% wt/vol organic matter (OM). Canopy cover was estimated 25, 32, 39, 46, 53, and 60 days after planting as the frequency of grass parts occurring under the vertical projections of 200 randomly located transect points. Canopy cover estimates were fitted to days after planting by the 3-parameter logistic function, using the NLIN procedure of SAS software (SAS Institute, Cary NC). Initial (day=0) canopy cover was assigned to day=0.5, to force convergence.

The logistic equation used was:

$$\mathbf{Y(t) = K / (1 + e^{(-(t-x)/b)})}$$

where:

Y(t) is the size or extent of the population at time (t),

K is the carrying capacity or maximum area extent,

t is time since initial planting,

b is a rate factor, and

x is a function of the shape.

The logistic function has slope:

$$\mathbf{dy / dt = r (1 - y/K) y}$$

where the exponential increase is:

$$\mathbf{r = 1 / b}$$

Growth of two bermudagrasses differing in growth rate.

To estimate the growth of two grasses together, it was assumed that growth of each grass was a function of the unfilled capacity for growth based on the sum of areas of two grasses:

$$dy_1 / dt = r_1 (1 - (y_1 + y_2)/K)\, y_1$$

$$dy_2 / dt = r_2 (1 - (y_1 + y_2)/K)\, y_2$$

where:

$$\text{Growth ratio} = r_1 / r_2$$

It was assumed that the two grasses were planted initially together in a total extent which was the same as the previous single-grass model, except the admixture rate, the content of the more aggressive grass in the mixture with the less aggressive grass, was varied from 0.001 to 0.01, across a range of growth ratios of 1.0 to 2.0. The simulation was run to completion (complete canopy coverage) for every combination of growth ratio and admixture rate, and proliferation (increase in contamination) was calculated as the proportion of the final admixture to the initial admixture:

$$\text{Proliferation} = \frac{\left(\dfrac{y_{1-\text{final}}}{y_{1-\text{final}} + y_{2-\text{final}}}\right)}{\left(\dfrac{y_{1-\text{initial}}}{y_{1-\text{initial}} + y_{2-\text{initial}}}\right)}$$

Likelihood of detecting a contaminant by random sampling.

If a field of turfgrass were determined as contaminated based on a single off-type sample, ß, the likelihood of falsely accepting the null hypothesis (that a field of turfgrass is homogeneous) is:

$$ß = (\text{purity})^{\text{number of samples}}$$

where:

purity = frequency of the intended clone

number of samples = number of samples required for a given ß

Alternatively,

$$\text{power} = 1 - ß$$

and,

$$\text{purity}^{\text{number of samples}} = 1\text{-power}$$

thus:

$$\text{number of samples} = \frac{\ln(1 - \text{power})}{\ln(\text{purity})}$$

$$\text{power} = 1 - \text{purity}^{\text{number of samples}}$$

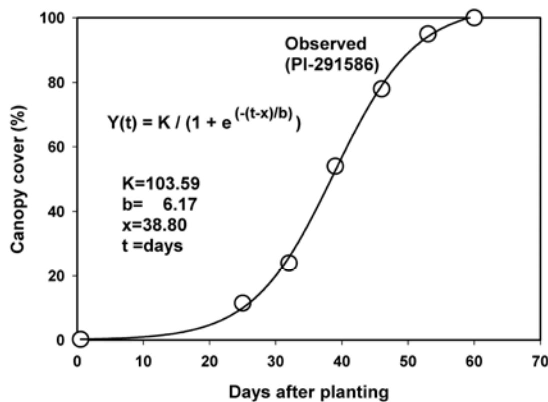
Contour plots were generated, demonstrating the number of samples required to reject a population of bermudagrass, based on the alternative hypothesis that it is not genetically homogeneous.

RESULTS

Growth of a single aggressive bermudagrass.

The logistic model (K=103.5886, b=6.1676, x=38.7966) effectively predicted ($r^2 > 0.999$) canopy cover of PI-291586 bermudagrass (Fig. 1). The exponential daily growth rate was r = 0.1621 and the initial cover at time zero was estimated as 0.001917 which was the value used in subsequent simulations.

Fig. 1. Growth in canopy cover of bermudagrass PI-291586 planted as 3.82 m^2 in a 1450 m^2 open field area fumigated with methyl bromide. The curve is based on the logistic model prediction.



Growth of two bermudagrasses differing in growth rate.

The combination of a slower spreading "cultivar" and a faster growing weedy contaminant is shown (Fig. 2) across a range of initial admixture rates, 0.001 to 0.01 and growth ratios, 1.0 to 2.0. Across a range of growth ratios, the more aggressive contaminant achieved a large expansion ratio, often several hundreds of times. For example, at a low admixture rate (0.1%), an even moderately aggressive contaminant (growth rate 1.5 times the intended slower growing cultivar) increased its proportion in admixture by contamination increase of 140 times in one propagation cycle.

Likelihood of detecting a contaminant by random sampling.

The number of samples required varied according to the desired power and the purity of the clonal population (Fig. 3). The sample number can be too large, in most instances, to be the only basis for a certification decision. For example, if one wishes to be 95% sure (power of rejection 0.95) that a field is 95% pure (actual purity of clonal population), it will require 59 samples. If one wishes to be 99% sure that a field is 99% pure, it will require 458 samples. To be 95% sure of detecting a 0.8% contaminant (field 99.2% pure), one would need to collect and analyze 373 samples, which is probably impractical. Therefore, random sampling alone would have a low likelihood of successfully finding contamination.

Fig. 2. Predicted contamination increase (final admixture / initial admixture) of a more competitive clone, as a function of growth ratio (growth rate of more aggressive clone:less aggressive clone) and initial admixture rate.

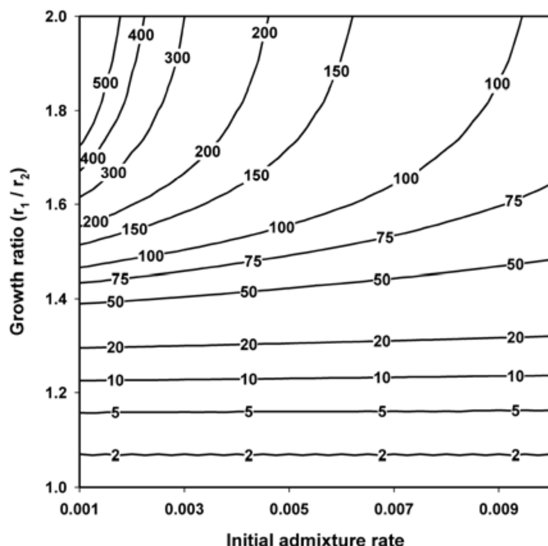
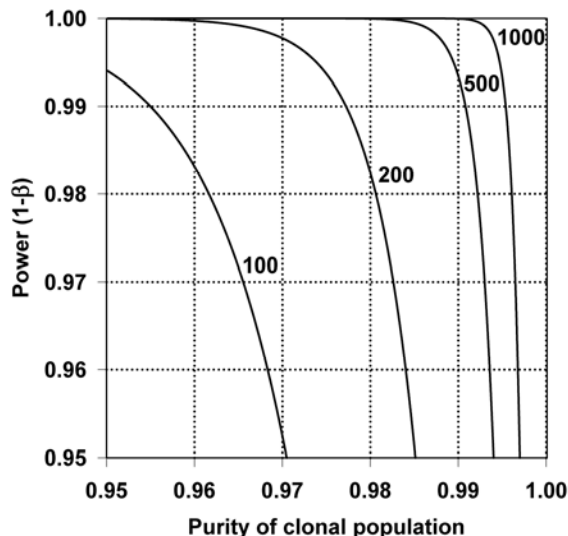


Fig. 3. Number of samples required to achieve different powers of rejection of contaminant admixture based on purity of the population, assuming that sampling is random and that the method of detection is perfect.



CONCLUSIONS

Genetic contamination, which can be a serious problem in the maintenance of quality turfgrasses, is expected to be greatly aggravated during expansion of grasses into largely vacant ground, as slight differences in growth rate would allow a more aggressive genotype to overwhelm a less aggressive genotype in filling the capacity. It is not apparent how or if the logistic growth model or some other equation could predict the competitive effects in a dense canopy where there is no unfilled capacity.

When genetic contamination of turfgrass cultivars might occur, some approach other than random sampling is necessary to assure genetic purity. The alternatives include an evidentiary trail to describe where a grass came from, ensuring that it can be traced back to a point of single-sprig propagation. For detecting genetic variants, visual sampling or other stratified approaches for *in situ* identification based on simple morphologic traits, especially growth habit, could support and complement DNA profiling, chromosome number, and grow-out tests in cases of suspected contamination.

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