Biological Control of *Poa annua* in Fairways

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Project Background:

Elimination of *P. annua* from golf course turf has been an objective of turfgrass managers and research personnel for decades. Its elimination from golf course fairways will enhance aesthetics, playability, save money and labor, and make the game a more positive experience for golfers. The use of a biological control to achieve this objective will be proactive environmental stewardship by the turfgrass industry.

Showing promise and warranting additional research is *Pseudomonas fluorescens* strain D7, a deleterious rhizosphere bacteria that has been registered (EPA Reg No. 71975-U, 71975-WA-001) by Verdesian Life Sciences primarily as a biological control (herbicide) in the agricultural sector for the control of downy brome, aka cheatgrass (*Bromus tectorum*). However, limited research has been done on turfgrasses. Kennedy et al. (2001) in a laboratory study showed D7 did not inhibit the growth or germination of tall fescue, perennial ryegrass, or Kentucky bluegrass. In a silt loam soil in the growth chamber, D7 did not inhibit the root growth or germination of tall fescue, but did inhibit the germination (but not root growth) of perennial ryegrass. In summary, D7 shows promise to provide biological control of weeds in the PNW, it was developed at WSU, has been under ongoing WSU research for over 20 years, and has been tested on numerous soils in the PNW.

Anecdotal observations have indicated that D7 might have some control of annual bluegrass (Jim Connolly, personal communication, 2015). Research is now needed to confirm, or dispute, the potential of D7 for the control of *P. annua* in turf. Also, following the initiation of the current project with D7, additional strains of *P. fluorescens* were reported that exhibited excellent selective control of *P. annua* in an agar bioassay, soil pot assay, and a meter-row field trial (Kennedy, 2016). To evaluate these promising additional strains, in late 2017, we initiated growth chamber and field studies (fairway and green at Palouse Ridge Golf Course at WSU).

Uniqueness of the study:

1. Potentially identify a biological control of *P. annua*.
2. A single application may provide long-term control (Fig. 1).
3. Biological control may eliminate *P. annua* seed presently in the seed bank over time, which is not possible with currently available herbicides.

Research Objectives:

1) Golf course study to determine the effect of timing and rates of D7 (fall only vs. fall + spring) on *P. annua* control in a mix *P. annua*/Kentucky bluegrass fairway over several years.

2) Fairway study to determine the effect of herbicide treatments (Tenacity + Xonerate and PoaCure) to initially knock down the *P. annua* population followed by D7 applications for long-term control. Biological controls typically start slow, giving essentially unacceptable control compared to chemical treatments; however, as the microorganisms increase over several years it is anticipated that biocontrol will eventually provide acceptable, continuous *P. annua* control (Fig. 1). Thus, with the combination (chemical + biological) there will initially be less *P. annua* to deal with and acceptable early chemical control followed by later biological control.

3) Evaluation of *P. fluorescens* strains, other than D7, to control *P. annua*. New objective added to the on-going project in late 2017.

Experimental Design & Methods:

Study 1 - evaluate timing and rates of D7 (D7 applied only in year 1) for *P. annua* control over three years. Field studies were initiated at Palouse Ridge Golf Course in late fall 2015 with applications of D7 to a Kentucky bluegrass/*P. annua* fairway.

D7 applied fall only. D7 was applied fall 2015 (11/16/15) at 0, 2, 10, or 30 g/acre. PoaCure was also applied at 1.26 fl oz/1000 ft^2 for a chemical treatment for comparison fall 2015 (11/16/15). Three applications of PoaCure were made fall 2017 (10/5/17, 10/19/17. and 11/2/17). Plots were evaluated for *P. annua* control and turfgrass quality during 2016 and 2017.

D7 applied fall + spring. D7 was applied fall 2015 (11/16/15) and spring 2016 (4/6/16). PoaCure was applied fall 2015 (11/16/15) and spring 2016 (4/6/16, 4/20/16, and 5/4/16). Three applications of PoaCure were made spring 2017 (4/15/17, 4/29/17, and 5/13/17) and 3 applications of PoaCure were made fall 2017 (10/5/17, 10/19/17, and 11/2/17). Plots were evaluated for *P. annua* control and turfgrass quality during 2016 and 2017.

Study 2 – Chemical + Biological to evaluate applications of herbicides (to initially knock back the *P. annua* population) followed by D7 applications over the top of the initial herbicide treatments. Herbicide treatments were: 1) PoaCure at 1.26 fl oz/1000 ft^2 in 3 applications spring 2016 (4/20/16, 5/4/16, and 5/18/16), 3

applications fall 2016 (10/5/16, 10/19/16/, and 11/2/16), and 3 applications summer 2017 (6/1/17, 6/15/17, and 6/28/17). Summer treatments were made instead of spring treatments in 2017 due to inclement weather, etc. during spring 2017. 2) Tenacity (4 fl oz/1000 ft^2) + Xonerate (1.4 fl oz/1000 ft^2) in 3 applications fall 2016 (9/7/16, 9/21/16, and 10/5/16) and 3 applications summer 2017 (6/1/17, 6/15/17, and 6/28/17). D7 was applied at 2, 10, or 30 g/acre fall 2016 (11/17/16) and 1, 5, or 15 fl oz/A fall 2017 (11/16/17); note, change in D7 formulation but equivalent rates. Plots were evaluated for *P. annua* control, turfgrass quality, etc. during 2016 and 2017.

Study 3 (new objective) – evaluation of *P. fluorescens* strains (other than D7) to selectively control *P. annua*. A combination of three *P. fluorescens* strains that have been reported (Kennedy, 2016) to have selective control of *P. annua* were applied to a fairway and a green at the Palouse Ridge Golf Course at WSU on 11/2/17. The field trial will consist of three application timings (fall 2017 only, spring 2018 only, and fall 2017 + spring 2018). Plots will be evaluated for several years for change in *P. annua* percentage, turfgrass quality, and other parameters as warranted. Also, currently underway are growth chamber studies evaluating formulations and rates on germination of six turfgrass species (*P. annua*, Kentucky bluegrass, creeping bentgrass, *P. trivialis*, fine fescue, and perennial ryegrass). Greenhouse studies will also evaluate application rates on emergence and growth of the same six species.

Results:

2016 (Year 1) -

Study 1. D7 applied fall 2015 only. D7 applied fall 2015 + spring 2016. The initial percentage of *P. annua* in the fairway was visually estimated at 30-35% (11/16/15). In 2016, there was no statistical difference in *P. annua* control in either the fall only or the fall + spring treatments. This was to be expected as the D7 applications have theoretically not yet had time to be effective and the PoaCure treatments were primarily applied in the spring 2016 just prior to the 5/26/16 rating date. However, it had been anticipated that the single fall PoaCure application on 11/16/15 would have had some reduction in *P. annua* (see below Study 2). It is anticipated that continued PoaCure applications in 2017 will show a reduction in *P. annua*.

Study 2. Chemical + Biological. The percentage of *P. annua* in the fairway was 35% (5/26/16), which was prior to application of D7 (11/17/16). The percentage of *P. annua* was again rated on 8/24/16 and 9/21/16. Unlike study 1, the reduction in *P. annua* compared to the check by PoaCure treatments was evident (Fig. 2). Compared to the initial rating (5/26/16) on 9/21/16 the *P. annua* in the check had increased 38% while *P. annua* in the PoaCure plots had a decreased 32% and in the Tenacity + Xonerate plots had decreased 11%. Tenacity + Xonerate and PoaCure treatments will continue in 2017.

2017 (Year 2) –

Study 1. D7 applied fall 2015 only.

On the final 2017 rating date (9/15/17), which was two years after the initial application of D7 there was no statistical differences in *P. annua* control by any D7 treatment (Fig. 3). Kennedy, Hansen, and Stubbs (Fig. 1) had observed approximately a 50% reduction in cheatgrass, a cool-season grass with a growth habit similar to *P. annua*, two years following an application of D7. To date we have seen little evidence of *P. annua* control with a single fall only application of D7; however, we will continue to monitor these plots in 2018.

PoaCure on 9/15/17 numerically showed 4% less *P. annua* than the check (Fig. 3), which indicates that the *P. annua* population was at least not increasing, as is the usual case. Three fall 2017 applications of PoaCure made were after the 9/15/17 rating date; thus, any control will not be evident till 2018. Also, in 2018 we plan on making three fall applications of PoaCure and we anticipate significantly more *P. annua* control by 2019.

Study 1. D7 applied fall 2015 + spring 2016.

Applying D7 in both the fall 2015 and the spring 2016 did not improve the control of *P. annua* (Fig. 4). There was no statistical difference between any of the D7 treatments and the check.

PoaCure on 9/15/17 numerically showed 11% less *P. annua* than the check (Fig. 4), which was better *P. annua* control than noted above in the fall 2015 only study (Fig. 3). Johnston and Golob (2015) have noted that spring applications of PoaCure, which these plots received in 2016 and 2017, in the Pacific Northwest have not provided the significant control of *P. annua* seen with fall applications. Three fall 2017 applications of PoaCure made in 2017 were after the 9/15/17 rating date and thus any control will not be evident till 2018. Also, in 2018 we plan on making three spring 2018 + three fall 2018 applications of PoaCure and we anticipate significant *P. annua* control by 2019.

Study 2. Chemical + Biocontrol.

Unlike study 1, the reduction in *P. annua* compared to the check by both Tenacity + Xonerate and PoaCure treatments was evident (Fig. 5). Compared to the 5/26/16 rating (36%) by fall 2017 (9/15/17) the *P. annua* in the check had increased to 56% while *P. annua* in the Tenacity + Xonerate plots (mean of three D7 treatments) had decreased to 17% and the PoaCure plots had a decrease to 22%. Thus, a reduction in *P. annua* was accomplished with chemical treatments.

D7 was applied in late fall (November) 2016 and 2017. Although there were no D7 alone treatments in the study, based on results from study 1, to date there does not appear to be additional control of *P. annua* by D7. There will be no additional application of D7, Tenacity + Xonerate, or PoaCure in 2018.

As anticipated, based on previous research (Golob and Johnston, 2015), the decrease in turfgrass quality due to the Tenacity + Xonerate application in fall 2016

and summer 2017 observed on 7/6/17 were completely gone by 9/15/17 (Fig. 6). Tenacity + Xonerate and PoaCure treatments had turfgrass quality ratings significantly better than the check (Fig. 6). The plots will be evaluated in 2018 and 2019 to determine if D7 treatments can maintain *P. annua* at these reduced levels, or perhaps enhance control as seen with cheatgrass control over time by D7 (Fig. 1).

Study 3. (new objective) Evaluation of additional strains of *P. fluorescens*. Field and laboratory studies were initiated late 2017. Field evaluation, on a green and fairway at Palouse Ridge Golf Course, of treatments applied fall 2017 will begin spring 2018.

Expected Benefits:

Potentially a *P. annua* biocontrol program for fairways will be identified. A single application of D7 (or the three additional *P. fluorescens* strains added to the trial in late 2017) may provide long-term control of *P. annua*. Initially the golf course industry will benefit the most, as with many new products and programs. The use of a biological control will provide turfgrass managers an additional option to the use of chemical control measures and will be hopefully seen as sound environmental stewardship by the industry and general public.

References:

Golob, C.T., and W.J. Johnston. 2015. Tenacity 4SC and Xonerate 70WDG in a spring-summer program for selective *Poa annua* control (post-emergence) in Kentucky bluegrass fairways. 2015 Washington State Weed Conference. Wenatchee, WA.

Kennedy, A.C. 2016. Pseudomonas fluorescens strains selectively suppress annual bluegrass (Poa annua L.). Biological Control 103:210-217.

Kennedy, A.C., B.N. Johnson, and T.L. Stubbs. 2001. Host range of a deleterious rhizobacterium for biological control of downy brome. Weed Sci. 49(6):792-797.

Kennedy, A.C., T.L. Stubbs, and J.C. Hansen. 2011. Microbial control of cheatgrass, jointed goatrass, and medusahead. www.fwaa.org/accounts/fwaa/data_documents/60/files/10b-dl-2011-12-13_130 p_kennedy.ann.pdf.

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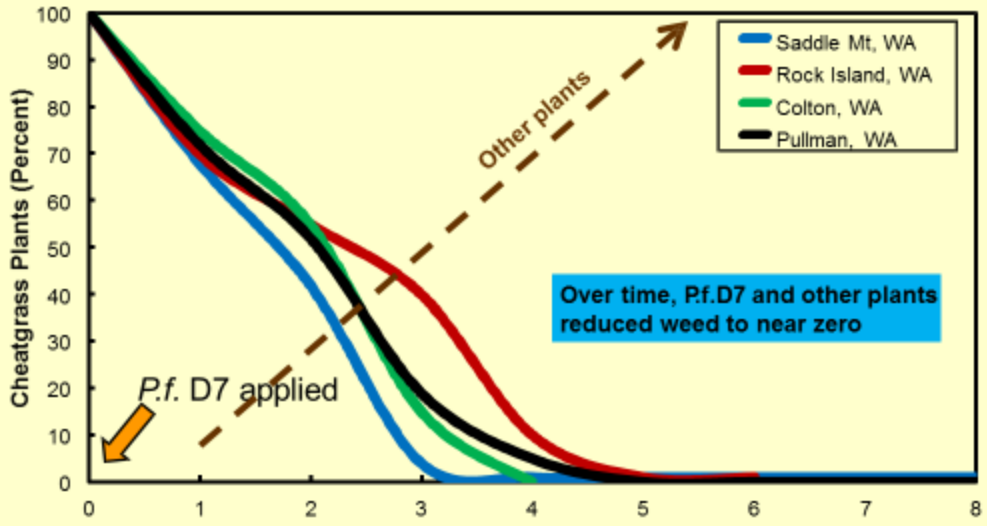
Fig. 1 Cheatgrass reduction over years following *a* D7 application.



5 replicate plots @ each site; P=0.05 for all years except t=0

Fig. 2. Percent change in *Poa annua* in a Kentucky bluegrass fairway

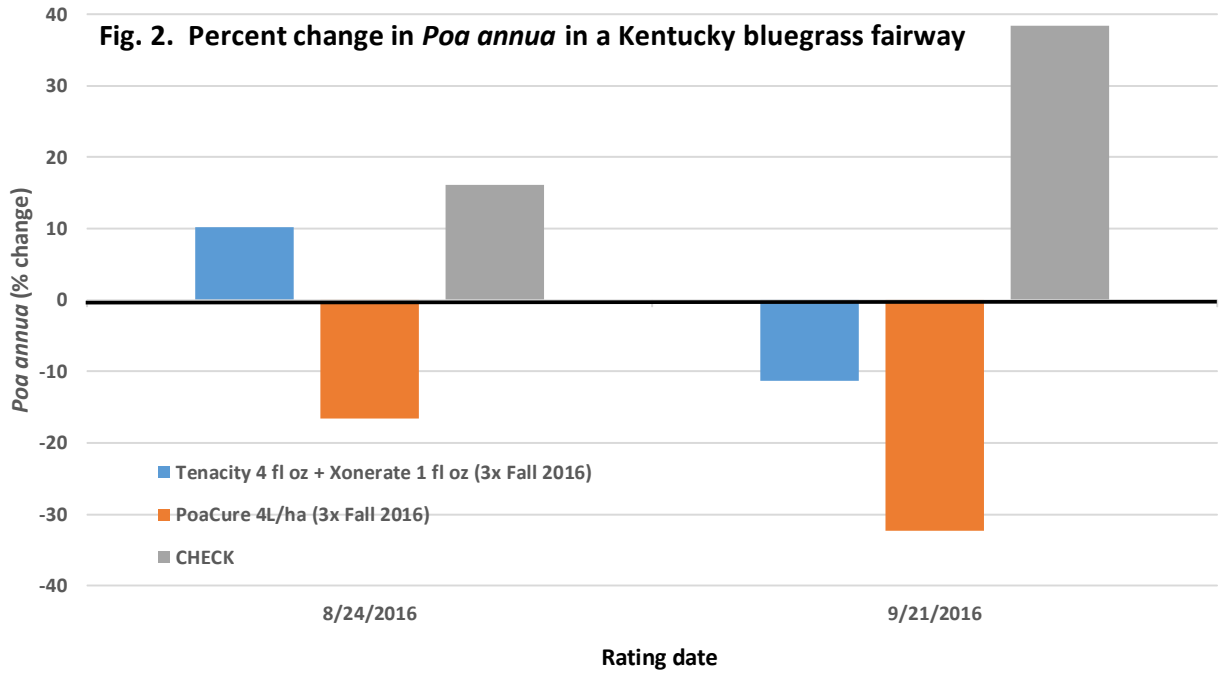
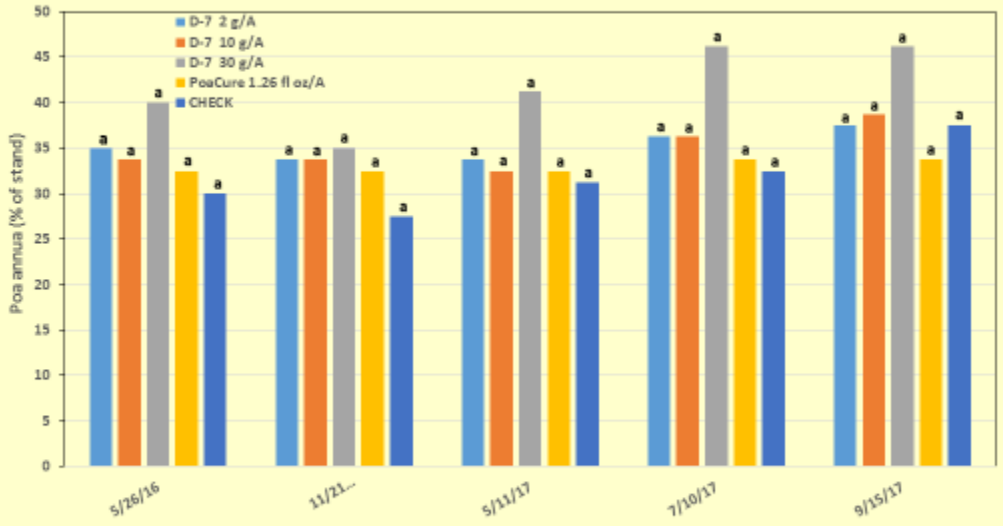


Fig. 3. D7 (Fall 2015 only) or PoaCure, Palouse Ridge G C

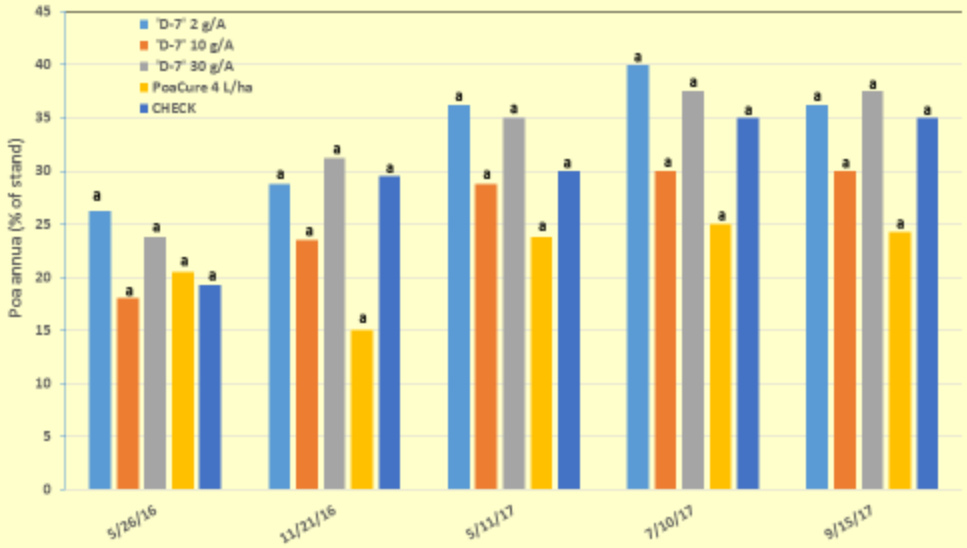


Fall 2015
D7 1 app
PC 1 app

Fall 2017
PC 3 apps

To do: PoaCure 3 apps Fall 2018

Fig. 4. D7 (Fall 2015 + Spring 2016) or PoaCure, Palouse Ridge G C



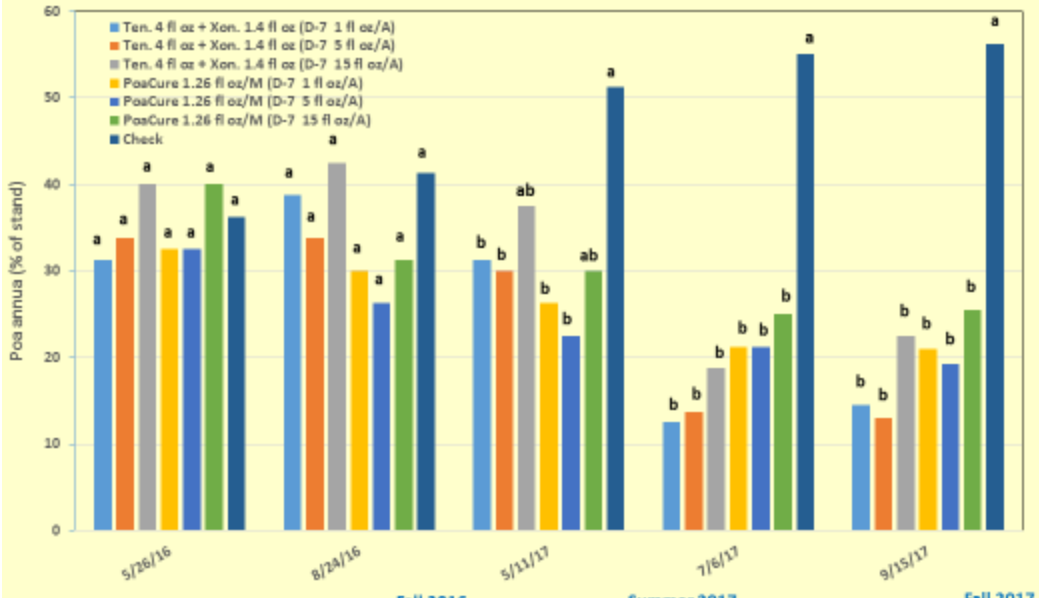
Fall 2015 Spring 2016 Spring 2017 Fall 2017

D7 1 app D7 1 app PC 3 apps PC 3 apps

PC 1 app PC 3 apps

To do: PoaCure 3 apps Spring 2018 and Fall 2018

Fig. 5. 3-year Chemical + Biocontrol, Palouse Ridge G C

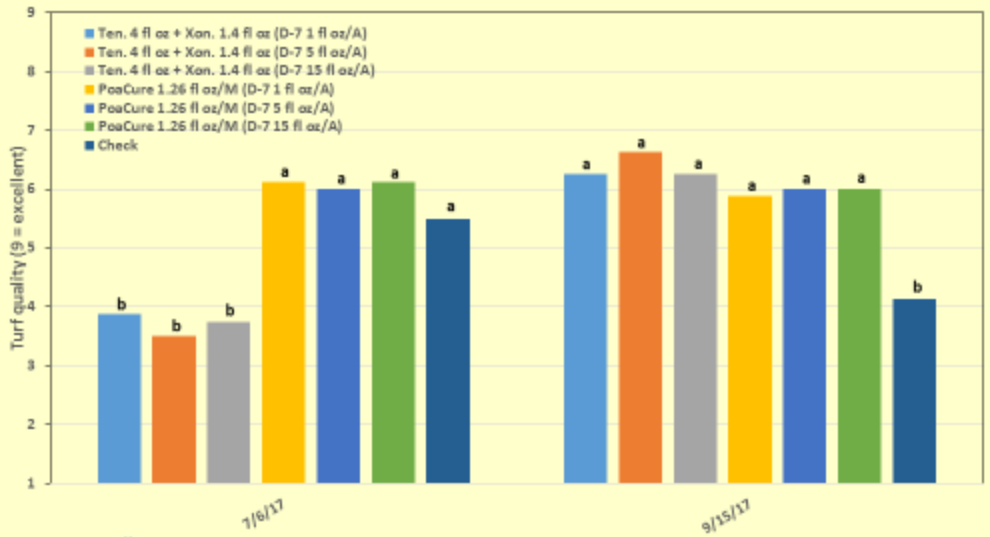


Spring 2016 Fall 2016 Summer 2017 Fall 2017

PC 3 apps D7 1 app PC 3 apps D7 1 app

PC 3 apps T+X 3 apps T+X 3 apps

Fig. 6. 3-year Chemical + Biocontrol, Palouse Ridge G C



Spring 2016
PC 3 apps

Fall 2016
D7 1 app
PC 3 apps
T+X 3 apps

Summer June 2017
PC 3 apps
T+X 3 apps

Fall 2017
D7 1 app