Selection of a perennial grain for seed productivity across years: Intermediate wheatgrass as a test species.

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<td>Keywords:</td>
<td>perennial grain, intermediate wheatgrass, selection, seed productivity, relative seed yield</td>
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Selection of a perennial grain for seed productivity across years: Intermediate wheatgrass as a test species.

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Corresponding author: Douglas J. Cattani, Doug.Cattani@umanitoba.ca
Abstract

Development of perennial grains is being promoted to aid in moving agriculture towards sustainable production. How long does it take to identify perennial yielding ability? Intermediate wheatgrass (*Thinopyrum intermedium*) nurseries were transplanted in 2011 (4,500 plants) and 2012 (1,000 plants) at Carman, Manitoba. Productive, healthy plants were harvested on a yearly basis. Intolerance to late frosts after spring regrowth initiation in 2012 (approximately 55% of the plants) and in 2014 (the entire 2012 nursery) led to these plants not being harvested. Mean yield plant$^{-1}$ was 37.25, 66.70 and 57.81 g for the 2012, 2013 and 2014 overall harvests, respectively. Individual plants showed divergent seed yielding patterns across years, especially with respect to the third harvest year. A number of plants were identified that had consistent relative seed yields over the three years of harvest for the 2011 nursery. Linear regression indicated that the first year of seed production was a poor indicator of relative yield potential over all three years ($R^2 = 0.238$) amongst the highest over-all yielding plants, with 2013 and 2014 having greater predictability. Variability in yield in the third reproductive year indicated that the third year is required to identify highly productive individuals for three consecutive seed harvests.

Key words: perennial grains, intermediate wheatgrass, seed productivity, selection, relative seed yield.
Introduction:

Perennial grains and perennial cropping systems, including mixed animal-plant (Bell et al. 2015) and agroforestry systems (Bardule et al. 2013), are being proposed as sustainable production systems (Bell et al. 2015). The inclusion of perennial crop species in production systems for use as food, feed and forage is dependent upon the successful development of the perennial grain species for agronomic production. Production and utilisation of perennial grains is based upon the premise that once established, the stand will be reproductive annually for a predictable period of time (Kantar et al. 2016; Vico et al. 2016). Duration of productivity will be dependent upon species, as individual plant species may have different reproductive life-spans (Pol et al. 2010), and upon the growth environment (Cattani et al. 2004).

Can we select for enhanced yield persistence in perennial grains? Vico et al. (2016) suggest that at least three years of seed production are required, coupled with a yearly doubling of biomass, and thus seed production, for perennial wheat and rice to provide similar yields to their annual counterparts. Bell et al. (2008) estimated that for perennial wheat to be an economically viable crop, it would need to reach a yield level of 60% of wheat in Australia.

Plant biomass appears to positively influence seed yield (Egli 2011), whether through greater percentage allocation (increasing harvest index (HI)) or by equal percent allocation to seed reproduction on a dry weight basis. Negative relationships have been shown in annuals with respect to the percentage of dry matter allocated to seed yield as biomass increases, however this still generally resulted in higher seed yields (Egli 2011). Within species differences for seed yield have been noted in perennial grasses through the maintenance of harvest index as the stand aged and was attributed to selection for seed yield in the growth environment of production (Cattani et al. 2004).
Components of yield, either for seed or for forage, need to be determined for selection criteria to be developed (Cattani et al. 2004; Boe and Beck 2008). Cattani et al. (2004) showed that selection for seed productivity within the region of seed production for a perennial grass species led to higher harvest index in years after the first seed harvest. This indicates that adaptation to the growth environment, as evidenced by reproductive productivity, needs to be considered for perennial species being developed for perennial grains as a first selection aim.

Selection for an increase in seed yield for annual grains utilizes harvest index (Casler and Brummer 2008). For perennial species, increasing harvest index may decrease overall life history duration via allocation to sexual reproduction versus perenniating structures and tissues (Zhang and Jiang 2000; Smaje 2015; González-Paleo et al. 2016). Aarssen (2005), extending the Fecundity Allocation Premium hypothesis, state that larger (by mass) species can have a greater range of variation of seed sizes (by mass) however as seed size increases, species are more likely to have a lower lifetime fecundity (i.e. larger, fewer seeds).

In perennial grasses, seed yield has been noted to decrease with stand age (Deleuran et al. 2013), with climatic factors impacting seed productivity (Mueller-Warrant and Rosato 2002a; 2002b) and with year to year variability in production also being reported (Marshall and Wilkins 2003; Cattani et al. 2004). Pol et al. (2010) found year x species interactions for seed yield in their study of seven perennial grasses in Argentina. Zhang et al. (2016) found that the second year of seed production was greater than the first in Thinopyrum intermedium. In western wheatgrass plants (Pascopyrum smithii) however, a correlation of $r = 0.83$ (P= <0.001) was found between first year seed yield and the second year seed yield (Robins et al. 2012). How long does this apparent yield correlation for a perennial plant remain between successive years and can we assume an infinite association?
If harvest index is fixed, larger plants have more resources to allocate to seed reproduction, thus giving a larger plant a reproductive advantage (Andrieu et al. 2007; Vico et al. 2016). In *Lesquellera mendocina*, second year seed yields were lower than the first year seed yield although percent dry weight allocation to seed (harvest index) was similar (Gonzalez-Paleo and Ravetta 2012). In the first year of seed production, a similar percentage of dry weight was allocated to seed productivity as in the annual *L. fendleri* (Ploschuk et al. 2005) as in *L. mendocina*. Ehlers and Olesen (2004) found that plant size, not age was important for seed yield for the iteroparous (seed production in successive years throughout life) *Corydalis intermedia*. Seed productivity of an individual would therefore appear to rely on plant size (Ehlers and Olesen 2004; Vico et al. 2016), however, in a crop stand individual plant size expression may be limited by intraspecific competition (Weiner et al. 2010). Jongejans et al. (2006) noted that enhanced persistence and seed productivity are not mutually exclusive and may be combined in a single plant. González-Paleo et al. (2016) found that there is a trade-off between longevity and seed productivity in *Physaria mendocina* (formerly *Lesquellera mendocina*). Selection for both seed yield and persistence in an individual may be possible, however most likely at a low rate within the population.

Plant reproductive type can influence the impact of seed production on future reproductive efforts. Hermaphroditic (perfect flowered) plants show less impact of on next season’s reproductive effort than gynodioecious (female) plants (Toivonen and Mutikainen 2012). Reduced likelihood of flowering is the primary impact on hermaphrodites versus less seed production in female plants (Toivonen and Mutikainen 2012). Timing and requirements for reproductive induction of perennials will also impact year to year seed yield potential (Thompson and Clark 1993; Heide 1994).
Agronomic practices will also impact seasonal productivity and overall long-term stand production. Soroka and Gossen (2005) found a species x residue treatment interaction with respect to seed yield the following year in three perennial grasses in western Canada. Post-harvest residue management or complete residue removal may be important for future seed productivity (Cattani et al. 1997; Soroka and Gossen 2005). Other factors, such as timing of fall frost occurrence and moisture can impact production such that year to year variability in yields is common (Cattani et al. 1997; Cattani et al. 2004; Mueller-Warrant and Rosato 2002a, 2002b;). Lee et al. (2009) speculated that year to year variability was due to favourable fall conditions (precipitation) allowing for adequate regrowth in intermediate wheatgrass (*Thinopyrum intermedium*) in one year, enhancing dry matter production and seed yield the following year. Timing of nutrient availability in perennials also appears to be important. Cattani et al. (1997) found that early fall fertilization provided a greater and more consistent impact on seed production the following year than fertilization in the spring of the year of production in *Agrostis stolonifera*. Therefore fertilization appears to have a growing season delay on reproduction in some perennial species as compared to annual species (Cattani et al. 1997; Burkle and Irwin 2009) due most likely to reproductive induction requirements (Heide 1994; Thompson and Clark 1989, 1993).

The objective of this study was to identify agronomically superior plants of intermediate wheatgrass through the year to year assessment of seed yield and its consistency for the development a perennial grain breeding and selection program.

**Methods and Materials:**

Seeds of intermediate wheatgrass were accessed from the USDA-NCRS GRIN collection and from Cycle 3 germplasm of the perennial grain breeding program of The Land Institute of
Salinas, KS (see Zhang et al. 2016). Approximately 4,500 plants, 50 plants from each accession (two rows of 25 plants), were transplanted into the field in the spring of 2011 at the Ian N. Morrison Research Farm of the University of Manitoba located at Carman, MB (N49°29’46.70, W98°2’43.64). The soil was a Rignold series, slightly acidic, loamy/clayey, imperfectly drained lacustrine loam (Canada-Manitoba Soil Survey, 1993).

Plants were transplanted into rows at 60 cm between plants within rows and 90 cm between rows. There were two rows for each accession family, randomized in two blocks. Inter-row cultivation was used to control weeds between rows and hand weeding for within row weed removal. Plants were clipped to 20-25 cm after harvest completion in 2012 and 2013. Fertilizer was applied in the fall after seed harvest and plant clipping at a rate of 30 kg ha⁻¹N using a 44-0-0 urea source.

Cycle 4 germplasm from the perennial grain breeding program of The Land Institute of Salinas, KS (See Zhang et al. 2016) was transplanted into the field in 2012, in a similar fashion. Climate data was collected at the Carman Research station and compiled on a daily basis for the cropping years studied (2011-2014). This data is found in Tables 1 and 2.

Plants were monitored for flowering initiation, with first flower being the day of the year when at least three inflorescences had visibly extruded anthers. Flowering observations were made three times weekly.

Individual plants were harvested for seed in 2012, 2013 and 2014 (≈1,700, 1,100 and 208 plants, respectively). Plants were harvested once seed shatter was noted or at the hard dough stage as per Berdahl and Frank (1998). Plants were cut, dried with forced air, and individually threshed. Seed was cleaned on a Westrup seed cleaner. Due to differences in free threshing between individuals (adherence of the lemma and palea), lemmas and paleas were removed through manual cleaning.
and all seed yields are reported on a bare caryopsis basis (Cox et al. 2010). The total seed sample was weighed. A sub-sample of seed was taken and weighed, (>25 g, where available). Lemmas and paleas were rubbed off using a rubber sandal on a rubber mat. Once all extraneous materials were removed, the sample was then re-weighed. Seed yields were expressed on a bare caryopsis weight basis (g) for all plants harvested. All Individual plant seed yields were then placed into 10 g seed yield classes to ascertain the distribution of seed yield across the harvested plants.

Yield data within individual years was then expressed on a relative basis for the 208 plants harvested in all harvest years. To remove seed yield variation due to the variability of growing years, a relative yield (RYld) value was calculated as follows:

\[ RYld = \left( \frac{Yld}{\text{mean Yld}} \right) * 100 \]

where, RYld (relative yield %) ((individual plant yield/mean yield for all plants)*100), Yld, yield of the plant in g for the year of harvest, and mean Yld being the mean yield for the 208 plants for the year of harvest. Seed yield plant\textsuperscript{1} and relative yield plant\textsuperscript{1} for each harvest year was regressed to the cumulative yield for the three years of harvest.

Analysis

Coefficient of variation (CV) for the relative yield of individuals was calculated only for the plants harvested in all three years. Regression of individual year seed yields to predict overall (cumulative) performance, both from an actual seed yield and a relative seed yield, of the plants harvested in 2014 was carried out using PROC REG in SAS 9.4 (Copyright (c) 2002-2012 by SAS Institute Inc., Cary, NC, USA).

Results:
Precipitation and temperature varied from year to year during this study (Tables 1 and 2). Temperatures for the early months in the growing season showed the greatest variability, with March mean daily temperatures ranging from 1.21°C in 2012 to -10.97 in 2014. Early season growth, if followed by severe frosts can be detrimental to survival. In 2012, after a warm March, low temperatures from April 9 through April 12, 2012, were -5.5, -6.5, -9.2 and -5.7 °C, respectively. These temperatures caused visible damage to approximately 55% of all plants and led to a range of effects on reproductive growth, from no reproductive tiller appearance, to a corona of reproductive tillers around a barren core, to two flushes of reproductive tiller emergence, with the second flush occurring at maturity of the first reproductive tillers. Plants that produced a corona of reproductive tillers were more likely to lodge. Lodged plants were not harvested in any year of the study. A lesser yet similar spring temperature pattern was seen in 2014 with a period of good growth temperatures followed by the consecutive low overnight temperature events on May 14-16, 2014. The lowest recorded temperatures were -3.5, -5.0 and -3.8 °C for May 14, 15 and 16, 2014, respectively. Cycle 4 plants all showed a lack of tolerance and therefore no seed was harvested in 2014 and all plants were removed from further testing. These plants had been selected for early flowering in Kansas (L.R. DeHaan, personal communication).

Distribution of seed yield across individual seed yield classes (gradations of 10 g plant⁻¹) can be found in Figure 1, where in 2012 many plants were in the lower seed yield classes. In 2013, with a reduction in plant number based on agronomic characterisitcs, and in 2014, with only relatively high yielding plants harvested, a normal seed yield distribution appears. Most low yielding plants in 2012 were low yielding in 2013.
Seed yield plant$^{-1}$ and mean seed yield year$^{-1}$ were variable from year to year with 2013 having the highest mean yield plant$^{-1}$, similar to the results of Zhang et al. (2016) in Minnesota and is demonstrated through a comparison of yearly mean seed yields for the 208 plants harvested in 2014 (Table 3 and Figure 2).

Regression of individual year seed yield plant$^{-1}$ on cumulative seed yield plant$^{-1}$ also demonstrates that 2013 had the highest seed yields (Figure 2), with 2012 and 2014 yielding less. Using actual seed yield values skews the importance of an individual year due to higher yields, possibly due to more a favorable production environment therefore relative yield was used to determine the impact of the individual years on overall seed yield plant$^{-1}$. Relative seed yields for 2013 showed a reduced $R^2$ value when compared to the actual yield value, indicating the utility of using relative yields (Figure 3). The first year of production, 2012, accounted for less than 25% of the variation in the cumulative actual yield or relative yield, making the first year of production the least reliable indicator of long-term yield plant$^{-1}$ in this study.

Relative seed yield indicated that the third year of productivity accounted for the greatest amount of variability in the cumulative relative seed yield (Figure 3). The adjusted $R^2$ values for 2013 and 2014 switched in the order of the explanation of seed yield (comparing actual to relative seed yield).

Yield stability was also looked at through the use of coefficients of variation (C.V.). Relative yield classes for the plants harvested in 2014, based on overall relative yield, were compared for their C.V.’s across harvest years. The six overall relative yield classes, having between 29 and 44 individuals, had C.V.s ranging from 0.56% to 76.70% (Table 4). The highest and lowest were found within the same relative yield class (90 to 100%). The two highest yielding plants overall had C.V.s of 9.53 and 8.14% (data not shown) for relative seed yield.
First flowering began on June 29, 2012, on July 2, 2013 and on June 30, 2014 with ranges for dates of first flowering across individuals being 14, 23 and 13 days for 2012, 2013 and 2014, respectively. Pearson correlation coefficients were determined between date of flowering and seed yield for all years. Correlation coefficients values were -0.156, -0.309 and 0.059 for 2102, 2013 and 2104, respectively.

**Discussion:**

Seed yield consistency is a major criterion for perennial grain development. Unlike annual species, perennial plants generally have a juvenile year (Heide 1994), coupled with exposure to year-long environmental conditions, both abiotic and biotic. Variability in growing season length and the impact of one year’s seed production on the next (Toivonen and Mutikainen 2012) complicates seed yield prediction in perennial species. The current study followed individual plants through three production years. Environmental conditions demonstrated an adaptation deficiency in over half of the planted materials before the first seed harvest. While the spring conditions of 2012 have been previously noted, another spring temperature fluctuation in Manitoba was shown to impact a relatively cold tolerant species, *Agrostis stolonifera* L. (Gusta et al. 1980), in both seed and turfgrass production (Cattani et al. 2000; Cattani et al. 2004). Extreme spring temperature fluctuations as noted here are not typical, however they may become more frequent as climate variability increases. Similar conditions were experienced in 2014 and this affected all of the 2012 planting of advanced materials from The Land Institute, Salinas, Kansas. However, the materials that had survived the spring of 2012 appeared to have been unaffected by the conditions in 2014. This may have been impacted by the harvest consisting only of the highest yielding materials from the previously two harvests. The early spring months of March and April in Manitoba had mean monthly temperatures below and above 0°C across
the study years (Table 2). Interestingly, 2013 had the coldest mean April temperature, with snow cover remaining until early May and produced the highest mean seed yields (Table 3). This was the second production year of the study, similar to results of Zhang et al. (2016). It is possible that it was not necessarily the year of seed yield (second) that is reflected in this result on seed productivity. Studies investigating the year of productivity versus growth conditions may help sort this out, however, as we will not be able to replicate previous year growth conditions (i.e. post-harvest regrowth conditions) across the study over time, we may not able to adequately answer this question. For example, the range in first flowering dates in 2012 and 2014 were similar while 2013 experienced a longer period of first flowering initiation due to climatic variables, and this will be next to impossible to control in the field.

Plants were removed from the trial for both seed yield related and non-seed yield related criteria. Bacterial leaf blight was evident across years and led to removal of some materials. Lodging, the inability of the stem to support the inflorescence, was another common removal factor.

As we are attempting to develop perennial grains that will remain productive in the growth environment for a number of harvests, we need to have an understanding of consistency in seed yield of individuals across years. Modern plant breeding relies on the selection of outstanding individuals for the trait(s) in question. Exceptional individuals are sought for use as parents for crop improvement (Zhang et al. 2016). Our selection of over 50 individuals from the 5,500 originally planted in 2011 and 2012 represents the top 1% of those tested. The third year of seed yield data allowed for the selection of plants that do not show a loss of relative yield over time (Figure 3). Interestingly, of the 208 plants harvested in 2014, the plants at the lower end of overall performance had their highest relative seed yield plant$^{-1}$ in their first seed production year while the highest grouping, in general had their highest relative seed yield plant$^{-1}$ in the third
year (Figure 3). Year 2 was the least likely to have the highest relative seed yield (Figure 3), although it was most likely to have the highest actual seed yield plant$^{-1}$ (Figure 2). Our attempt to convert seed yields on individual plants to crop yield estimates indicates that we are in the 1500-2000 kg ha$^{-1}$ range. These are estimates only and dependent upon the agronomic practices we imposed, but we feel are within our target range for an initial release.

Populations consist of diverse individuals, especially in obligate outcrossing species such as *Thinopyrum intermedium* (DeHaan et al. 2014). We made an effort to ensure that we had materials that were from different backgrounds. In general, the improved populations (The Land Institute, TLI) were best suited to perennial grain production. However, there was great divergence in all materials, especially with respect to the third seed production year, both in actual and relative terms. In general, the growth environment exerted selection pressure on the materials outside of the growth conditions under which they had been selected (TLI) or collected from (USDA-GRIN).

Long-term yield stability can only be confirmed by long-term testing. The current study demonstrated that seed yield in a herbaceous perennial grass species was inconsistent across the population tested. Seed yield is comprised of numerous plant characteristics (Dewey and Lu 1959) and is subject to yield component compensation (Dewey and Lu 1959; Dofing and Knight 1992). These factors may exert influence both across and within years (Cattani et al. 2004).

The top two plants overall were never the highest yielding plant in any year, however they were consistent (relative yield) when compared to the other plants harvested in 2014 for each of the production years. These values were 158%, 170% and 191% for plant 1 and 160%, 170% and 183% for plant 2, for 2012, 2013 and 2014, respectively. The highest yielding plant in 2012 had a continuous drop off in yield across years, both in relative yield (%) and actual seed yield (g).
terms (Figures 2 and 3). The relative yields for this plant were 180%, 70% and 40% for 2012, 2013 and 2014, respectively. Long-term testing of individuals is therefore necessary to identify exceptional plants for the seed yield potential required for perennial grain development. Traits that are consistent across years may be identified in a single harvest, e.g. seed size (DeHaan et al. 2014). Other traits that influence seed yield, e.g. plant area achieved by a plant in space-planted nurseries, may impact total seed yield plant$^{-1}$. Our design attempted to limit this by close interplant spacing within rows between individuals.

Conclusions:

Unlike annual plants, perennial plants must be tested across the number of years that they will be expected to be reproductively active. Selection based upon short-term testing will most likely prove to be less efficient than longer term testing. Selection success due to long-term testing will be enhanced in growth environments that have limitations, especially climate related factors. when introducing perennial grains for commercial use, it is incumbent upon breeders to accurately select for long-term yield potential for producer acceptance. Our results indicate that for the development of herbaceous perennial food crops for western Canada evaluation of seed yield consistency should be through at least three consecutive reproductive years.

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I would like to thank the anonymous reviewers for their helpful comments on the manuscript. Funding for this research was provided by grants and aid from Manitoba Agriculture, Food and Rural Initiatives – ARDI and Growing Forward I, the Manitoba Wheat and Barley Growers Association, the University of Manitoba and the Department of Plant Science, University of Manitoba. I would like to thank all research technicians and summer research associates that participated in this program.
Literature Cited:


List of Figures:

Figure 1. Seed yield plant\textsuperscript{−1} distributions for 2012(a), 2013(b) and 2014(c) harvests of intermediate wheatgrass at Carman, MB.

Figure 2. Relationship of individual yearly seed yield (g plant\textsuperscript{−1}) to cumulative actual seed yield plant over the three years of harvest for the plants harvested in 2014 of intermediate wheatgrass at Carman, MB. Filled diamonds (2012 harvest); filled squares (2013 harvest); and filled triangles (2014 harvest).

Figure 3. Relationship of relative seed yield (% of mean) to the three average relative yield for 2012, 2013 and 2014 of intermediate wheatgrass at Carman, MB. Filled diamonds (2012 harvest); filled squares (2013 harvest); and filled triangles (2014 harvest).
Table 1. Monthly precipitation (mm) for Carman, MB in 2011-2014 and thirty year average.

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Table 2. Mean monthly temperatures (°C) for 2011-2014 and the preceding 20 year mean monthly temperatures at Carman Manitoba.

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Table 3. Mean seed yield plant\(^{-1}\) (g) and standard deviation (s.d.) (in parenthesis) for all plants harvested and for the 208 plants harvested in 2014 for the 2012, 2013 and 2014 seed harvests.

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g plant(^{-1}) (s.d.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All plants harvested</td>
<td>37.25 (20.70)</td>
<td>66.70 (30.37)</td>
<td>58.81 (23.51)</td>
</tr>
<tr>
<td>Seed yield range</td>
<td>0.30 – 121.92</td>
<td>1.86 – 177.21</td>
<td>9.82 – 132.70</td>
</tr>
<tr>
<td>2014 harvested plants</td>
<td>67.64 (13.86)</td>
<td>102.78 (23.98)</td>
<td>58.81 (23.51)</td>
</tr>
<tr>
<td>Seed yield range</td>
<td>35.33 – 121.92</td>
<td>49.11 – 177.21</td>
<td>9.82 – 132.70</td>
</tr>
</tbody>
</table>
Table 4. Mean coefficient of variation (C.V.) for individual relative yield class (compared to the mean), number of individuals within each class (n) and the range of C.V.’s for each class grouping.

<table>
<thead>
<tr>
<th>Relative Yield Class</th>
<th>Mean C.V.</th>
<th>n</th>
<th>C.V. range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 80%</td>
<td>34.58</td>
<td>32</td>
<td>9.67 – 65.16</td>
</tr>
<tr>
<td>80% to 90%</td>
<td>16.91</td>
<td>38</td>
<td>2.87 – 35.06</td>
</tr>
<tr>
<td>90% to 100%</td>
<td>17.72</td>
<td>44</td>
<td>0.56 – 76.70</td>
</tr>
<tr>
<td>100% to 110%</td>
<td>19.50</td>
<td>35</td>
<td>1.29 – 41.10</td>
</tr>
<tr>
<td>110% to 120%</td>
<td>21.35</td>
<td>29</td>
<td>5.53 – 45.60</td>
</tr>
<tr>
<td>Greater than 120%</td>
<td>23.62</td>
<td>31</td>
<td>5.91 – 51.36</td>
</tr>
</tbody>
</table>
2012: 
\[ y = 1.626x + 118.25 \]  
\[ R^2 = 0.2378 \]

2013: 
\[ y = 1.6478x + 58.864 \]  
\[ R^2 = 0.7316 \]

2014: 
\[ y = 1.5826x + 136.74 \]  
\[ R^2 = 0.6485 \]
2012
\[ y = 0.5016x + 49.842 \]
\[ R^2 = 0.2371 \]

2013
\[ y = 0.7045x + 29.552 \]
\[ R^2 = 0.6066 \]

2014
\[ y = 0.4487x + 55.129 \]
\[ R^2 = 0.7476 \]