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Displacement of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), Biotype 1 in Colorado by Russian wheat aphid biotypes virulent to the wheat resistance gene *Dn*4

Displacement of Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), Biotype 1 in Colorado by Russian wheat aphid biotypes virulent to the wheat resistance gene *Dn*4

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Abstract

The Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov), is an economically important pest of winter wheat and barley. This invasive pest has caused economic damage in excess of a billion dollars since it was first identified in the United States in 1986. Numerous management tactics exist for control of RWA such as the use of resistant cultivars, adjusting timing of crop planting, and the application of pesticides. The use of resistant cultivars is likely the most economically and ecologically attractive control method because planting resistant cultivars eliminates the need for treatment with insecticides. Unfortunately, the effectiveness of all commercially available resistant cultivars was compromised in 2003 when a new RWA biotype, virulent to the *Dn*4 gene (the gene responsible for resistance in virtually all commercially available resistant wheat cultivars), was discovered in SE Colorado. Since 2003, several additional RWA biotypes have been discovered, each of which is virulent to the *Dn*4 gene. Survey work was undertaken in Colorado from 2004 through 2008 to determine the extent of the spread of RWA biotypes virulent to *Dn*4. A Markov transition matrix was developed to predict the spread of *Dn*4 virulence and displacement of RWA Biotype 1 (susceptible to *Dn*4 resistance). RWA biotypes virulent to *Dn*4 appear to be rapidly displacing RWA Biotype 1. Survey results and transition matrix predictions indicate that wheat cultivars carrying the *Dn*4 resistance gene will have minimal value for RWA control by the year 2010.

Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), is an economically important pest throughout small-grain producing areas west of the 100th meridian (Elliott et al. 1998, Morrison and Peairs 1998). Since its introduction in 1986 into the United States, this aphid has caused economic damage in excess of a billion dollars (Webster et al. 1994, Morrison and Peairs 1998). Symptoms of plant damage include leaf streaking or chlorosis, leaf rolling, and under heavy aphid pressure, plant death (Walters et al. 1984, Webster et al. 1987). Numerous management tactics exist for control of RWA including the use of resistant cultivars, adjusting planting timing, controlling volunteer wheat (which, if uncontrolled, can act as important oversummering habitat) and the application of pesticides (Walters et al. 1984, Peairs 1990, Peairs et al. 2006). Biocontrol efforts have not been shown to have high efficacy (e.g., Randolph et al. 2002). However, research examining natural enemy efficacy as a complex, as contrasted with efficacy of individual species, is limited. Resistant cultivars are likely the most economically and ecologically attractive control method. Planting resistant cultivars eliminates the need for treatment with insecticides and thus reduces the overall economic impact even when RWA abundance is high. To aid in the development of resistant cultivars, Quick et al. (1991) tested numerous wheat genotypes for resistance. They found the wheat genotype, PI 372129, to be resistant to the original RWA introduction. The PI 372129 wheat genotype is the source of the *Dn*4 gene which was bred into many RWA resistant wheat lines. Unfortunately, the effectiveness of all commercially available resistant cultivars (inclusive of wheat cultivars with the *Dn*4 gene, as well as cultivars with the *Dn*y gene such as Stanton) was compromised in the spring of 2003 when a new RWA biotype (designated as RWA2) was discovered in SE Colorado (Haley et al. 2004). RWA2 is virulent to wheat with the *Dn*4 gene and has virtually eliminated resistant wheat cultivars as a management tool. Since the discovery of RWA2, additional biotypes have been discovered in Colorado, Texas and Wyoming (Haley et al. 2004, Burd et al. 2006, Weiland 2006). The mechanism behind this increase in genetic diversity and evolution is not yet understood. Dixon (1985) suggests that there may be multiple mechanisms for genetic recombination during parthenogenesis and these mechanisms may be responsible for some of the observed changes in biotypic diversity. Additionally, while no male RWA have been found to date, sexual selection may be occurring. Biotypic variation and evolution has been observed in other aphid species. For example, biotypic variation in greenbug was first described in 1961 (Wood 1961). Since then, the original greenbug biotype (greenbug Biotype A) can no longer be found in field populations and is believed to have gone extinct (Porter et al. 1997).

New wheat cultivars resistant to known RWA biotypes are on track for release possibly as soon as 2010 (Haley, unpublished data). These new cultivars will utilize the rye-derived *Dn*7 gene (Marais et al. 1994, Peng et al. 2007). While this source of resistance allows for increased yield under aphid feeding pressure, it has also been linked to wheat end use quality issues (e.g., dough stickiness during processing) (Marais et al. 1994). Significant breeding efforts have been undertaken to circumvent these quality issues.

Relying on one management strategy, even resistant cultivars, places high selection pressure on the aphid to overcome that strategy (Souza 1998). Thus, RWA is likely to eventually overcome whatever new sources of resistance are incorporated into wheat, especially if resistant cultivars are relied upon as the primary control tactic. While development of resistant cultivars continues, the level of resistance to existing sources of resistance needs to be monitored to determine the value of current resistance genes and the cultivars using them. Yield damage was found to be linearly proportionate to the mixture of resistant cultivars deployed (Randolph et al. 2007). Therefore, where biotypic variation exists, resistant cultivars should have value that is relatively proportionate to the ratio of aphids that are virulent and susceptible to the resistance gene.

Our goal was to determine the distribution of RWA biotypes virulent to the *Dn*4 gene (hereafter referred to as Virulent) and susceptible to this resistance gene (i.e., RWA Biotype 1, designated as RWA1). Specifically, survey work was conducted annually from 2004 through 2008 to determine virulence of field-collected Russian wheat aphid to resistance sources used by Colorado State University's Wheat Breeding Program. In 2005, Puterka et al. (2007) examined RWA biotypic diversity in a study of 365 RWA isofemale lines from 98 sites across Oklahoma, Texas, New Mexico, Colorado, Kansas, Nebraska, and Wyoming. Their study found approximately 28% RWA1 and approximately 72% RWA2 (no other biotypes were observed). RWA biotypes are currently defined by differential virulence to a set of 24 wheat and barley genotypes (WERA-66 2005) caused by aphid feeding damage. For example, Randolph et al. (unpublished data) developed biotype virulence profiles for the seven of the eight named RWA biotypes using a set of 24 plant differentials.

Materials and Methods

Differentiating Biotypes. For logistical purposes, we used a limited set of three plant differentials to determine whether collected survey samples (i.e., field collected RWA) were susceptible or virulent to the *Dn*4 gene. Two cultivars 'Yuma' (a susceptible wheat cultivar) and 'Yumar' (a cultivar containing the *Dn*4 gene, resistant to RWA1) were used as plant differentials. A third plant differential, STARS 02RWA2414-11, resistant to all known North American RWA biotypes, contains the *Dn*7 resistance source (Peng et al. 2007). This line, although not yet commercially available, was used because the next cultivar release is expected to utilize this resistance source. Three seeds of each cultivar were planted in 5" pots and watered as needed. After emergence, wheat tillers would be removed until only one tiller of each plant differential remained (i.e., tillers were removed to provide approximate biomass equivalence between differentials). Survey samples were isolated to one pot. Specifically, per survey sample, ten RWA were placed on each of the three plant differentials when tillers were at the two to three leaf stage (Zadoks 21 or 22 (Zadoks et al. 1974)). Organza cloth was placed over pots to isolate the aphid sample colonies. Damage to plant differentials was rated when the susceptible cultivar Yuma rated an 8 or 9 on the 1-9 scale (Webster et al. 1987, Burd et al. 1993) for chlorosis (i.e., dead or nearly dead). At this point, if the resistant cultivar Yumar showed feeding damage symptoms (indicating that the aphid had overcome the *Dn*4 resistance) then the aphid sample was considered Virulent. Additionally, if Yumar showed feeding damage symptoms before the susceptible was rated, the aphid sample was considered Virulent. Collected aphid samples that include a mix of both RWA1 and biotypes resistant to *Dn*4 will elicit a Virulent result using plant differentials. Therefore, a Virulent result is equivalent to stating that a portion of the aphids sampled were virulent to *Dn*4 at the sample location. However, since a mixed population of aphids (multiple biotypes) would elicit a Virulent result, such a result does not preclude RWA1 from also being at the sample location.

Survey sample collection was designed to collect samples per wheat producing county throughout Colorado based on 2004 production estimates. That is, counties producing large amounts of wheat were sampled more than counties producing little wheat.

Quantifying spread of virulence to the *Dn*4 resistance gene using a Markov transition matrix. The spread of biotype(s) virulent to *Dn*4 (likely synonymous with the displacement of RWA1) can be quantified using a Markov transition matrix (e.g., Doak 1992). The Markov transition matrix technique looks at transitions between several states. We examined three state variables:

1. All sampling sites within a county were RWA1 (Biotype 1)

2. All sampling sites in a county were Virulent (Virulent)

3. Sampling sites test as both RWA1 and Virulent in the county (Both)

For example, in 2004, Adams County, CO had some sites that tested as RWA1 and some sites that tested as Virulent. In 2005, all of the sites sampled within Adams county, CO tested as Virulent. Therefore, Adams County changed from a "Both" state to a "Virulent" state. All state variables are based on countywide data. Nineteen Colorado counties were sampled in both 2004 and 2005. Using a Markov transition matrix allows us to project the state variables across future time steps. Specifically, predictions were generated for each of the state variables from 2006 onward. The number of samples obtained within each county changed between years. However, this variation was not incorporated into the transition matrix.

Results and Discussion

***Diuraphis noxia* survey results.** No RWA were found that damaged the most advanced RWA resistant wheat line (STARS 02RWA2414-11, containing resistance imparted by the *Dn*7 gene) from Colorado State University's Wheat Breeding Program. This result provides encouragement that upcoming releases of new wheat lines utilizing the *Dn*7 resistance source will be resistant to field populations of this pest in Colorado.

Remaining results will concentrate on discussion of RWA1 contrasted with aphids virulent to *Dn*4. Survey results indicate that aphids virulent to *Dn*4 appear to have effectively displaced RWA1 (Figures 1-5). Aphid samples were collected from 84 Colorado wheat fields in 2004. Of the sampled locations in 2004, 48% tested as RWA1 (40 of the 84 sampled sites), and 52% of the locations tested as Virulent (Figure 1). Aphid samples were collected from 122 Colorado wheat fields in 2005. Of the sampled locations in 2005, 18% tested as RWA1 (22 of the 122 sampled sites), and 82% of the locations tested as Virulent (Figure 2). Aphid samples were collected from 118 Colorado wheat fields in 2006. Of the sampled locations in 2006, 7% tested as RWA1 (8 of the 118 sampled sites), and 93% of the locations tested as Virulent (Figure 3). Aphid samples were collected from 51 Colorado wheat fields in 2007. Of the sampled locations in 2007, 4% tested as RWA1 (2 of the 51 sampled sites), and 96% of the locations tested as Virulent (Figure 4). Aphid samples were collected from 49 Colorado wheat fields in 2008. Of the sampled locations in 2008, 2% tested as RWA1 (1 of the 49 sampled sites), and 98% of the locations tested as Virulent (Figure 5).

Sample numbers decreased in 2007 and 2008 because of decreased abundance of RWA. Scouting time necessary to obtain samples was dramatically increased during these years as compared to 2004 through 2006.

2005 were similar to those found by Puterka et al. (2007). That is, our results indicate that 82% of samples taken in 2005 were Virulent while Puterka et al. (2007) found 72% of samples to be Virulent. Because RWA2 appears to have originated within Colorado, using an assumption of contiguous spread from the origin, one would expect to have greater frequency of RWA2 samples encountered near the origin. Therefore, samples taken within Colorado would be expected to have a larger proportion of Virulent results than samples taken from across a larger extent.

While displacement of RWA1 seems apparent, it is important to remember that collected samples may have populations of mixed biotypes and if any biotype other than RWA1 were present in the sample, then the sample will appear to be Virulent. That is, a Virulent result from differentials is equivalent to stating that aphids virulent to *Dn*4 were collected at the location, but that we cannot rule out that RWA1 also exists at that location. However, results of isofemale line testing from Puterka et al. (2007) suggest that Virulent results are likely RWA2.

Virulence ratings are affected significantly by environment, with more severe symptom expression typically observed in the greenhouse than in the field. Additionally, aphids held in the greenhouse are protected from a variety of biotic and abiotic mortality factors that operate under field conditions. Our survey methods, therefore, produce “worst case” results. Because of this, it is possible that populations of *D. noxia* that show intermediate virulence to wheat varieties resistant to RWA1 in the field may be rated virulent in survey tests, and that such varieties actually may provide limited, but useful, protection against other biotypes. Many biotypes (i.e., *D. noxia* biotypes: RWA4, RWA5, RWA6, RWA7) showed intermediate virulence to the Yumar cultivar (Randolph et al. unpublished data).

Markov transition matrix results predicted approximately complete displacement of RWA1 by Virulent aphids by 2010 (Figure 6). However, field results show a more drastic shift towards displacement than predicted by the transition matrix. For example, in 2006, 76% of counties sampled tested as Virulent. However, the transition matrix predicted that 61% of the observed counties sampled would test as Virulent.

Results indicate a displacement of RWA1 is occurring within Colorado with prevalence expected drop to minimal levels by 2010. Fortunately, no virulence has been found to a new source of resistance currently being incorporated into winter wheat breeding material by the Wheat Breeding Program at Colorado State University. However, the quick displacement of RWA1 by multiple resistant biotypes indicates a need for a robust pest management strategy, inclusive of sound resistance management when new cultivars become available.

Biotypic variation and the displacement of RWA1, which has been glimpsed through survey results, are indicative of *D. noxia* evolution. Increased within-species genetic variation has been associated with increased species fitness (Reed and Frankham 2003). That is, increased biotypic variation implies that *D. noxia* may have increased abilities to adapt to novel wheat cultivars. Therefore, it is unlikely that *D. noxia* will not eventually overcome resistant sources present in our most advanced lines. However, intermediate stages of virulence to *Dn*4 exist in the North American *D. noxia* population, which argues that *Dn*4 likely still holds some value years after its introduction. If history repeats itself then *Dn*7 may have value for many years to come.

Figure 1: 2004 RWA biotype survey. Cross-hatched counties indicate no reported wheat acreage harvested in 2004. Wheat production increases within counties as they become lighter on the grayscale.

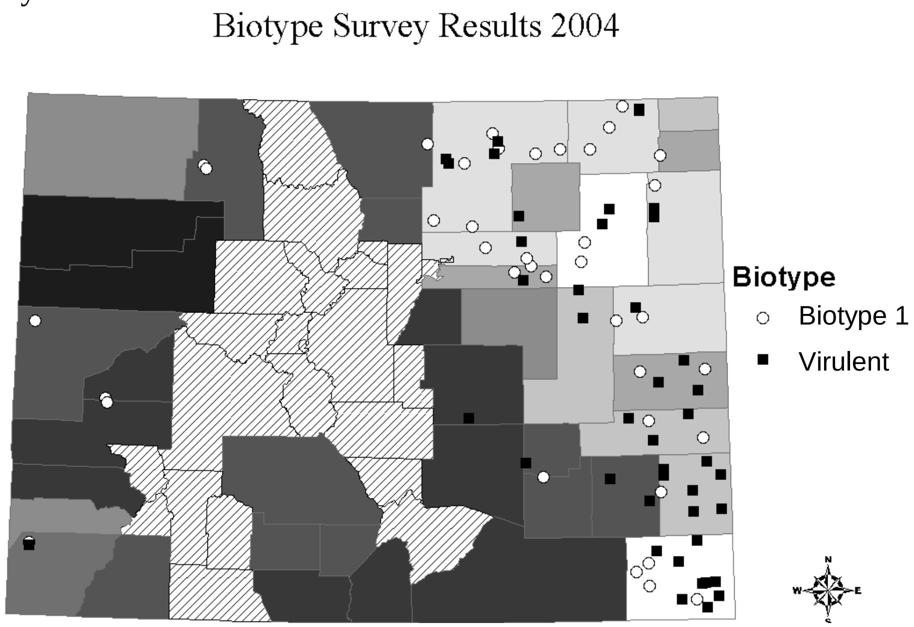


Figure 2: 2005 RWA biotype survey. Cross-hatched counties indicate no reported wheat acreage harvested in 2004. Wheat production increases within counties as they become lighter on the grayscale.

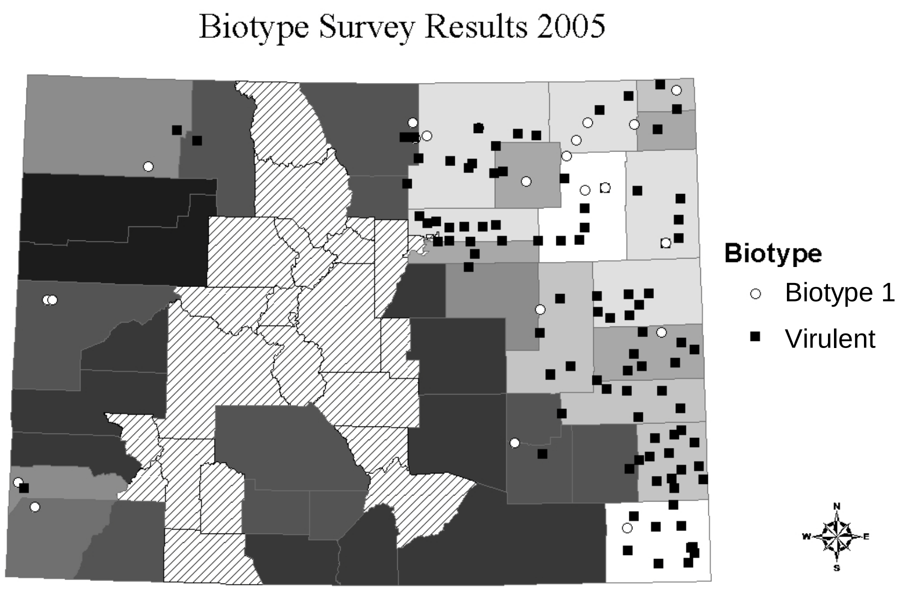


Figure 3: 2006 RWA biotype survey. Cross-hatched counties indicate no reported wheat acreage harvested in 2004. Wheat production increases within counties as they become lighter on the grayscale.

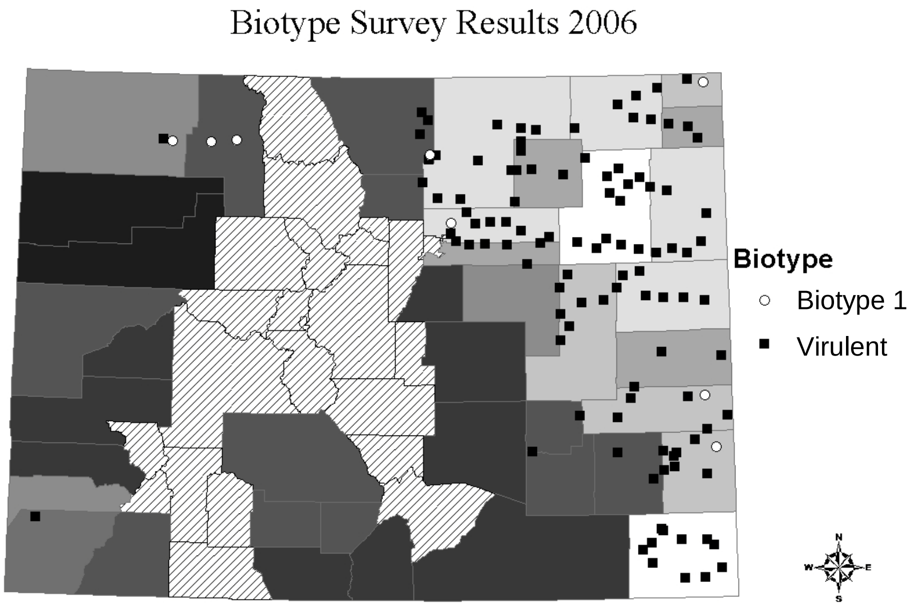


Figure 4: 2007 RWA biotype survey. Cross-hatched counties indicate no reported wheat acreage harvested in 2004. Wheat production increases within counties as they become lighter on the grayscale.

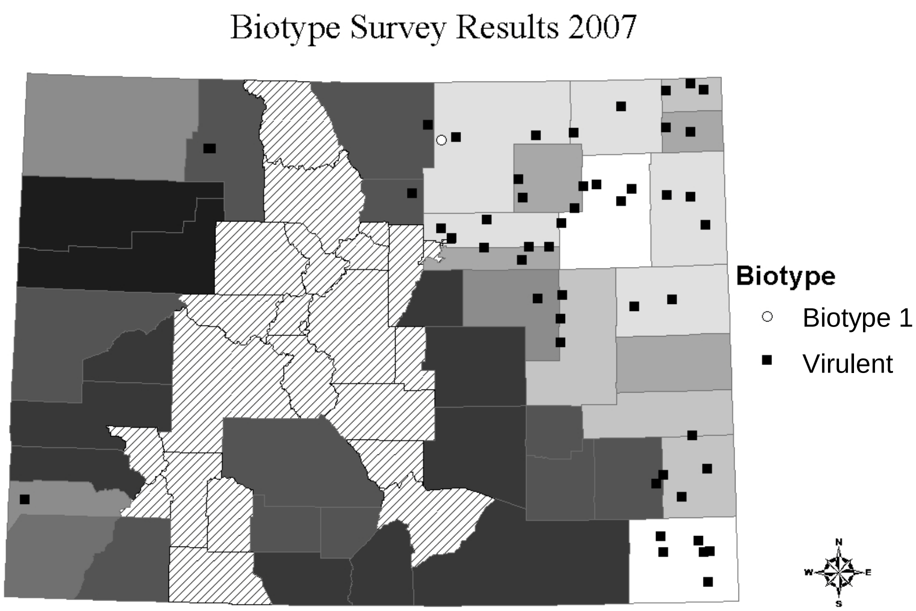


Figure 5: 2008 RWA biotype survey. Cross-hatched counties indicate no reported wheat acreage harvested in 2004. Wheat production increases within counties as they become lighter on the grayscale.

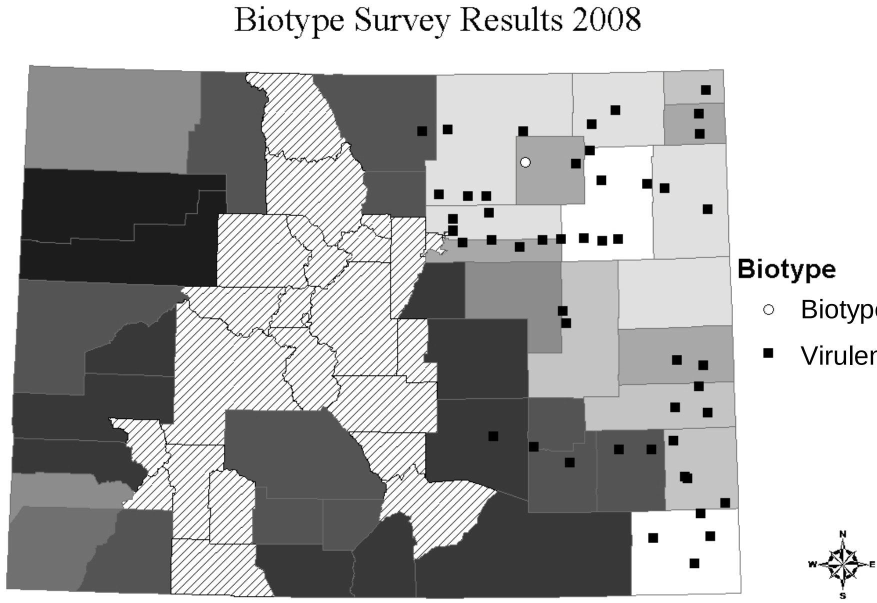
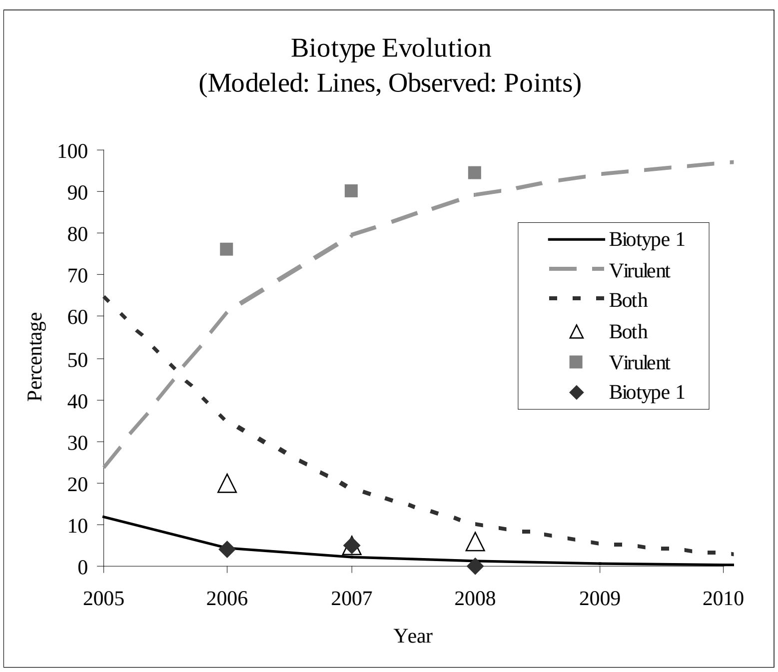


Figure 6: Markov transition matrix. Points (squares, diamonds and triangles) indicate observed values. Lines indicate Markov transition matrix predictions.



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