THESIS

MONTANE ENVIRONMENTS AS A SOURCE OF BIOTYPIC DIVERSITY IN RUSSIAN WHEAT APHID, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), IN COLORADO

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ABSTRACT

MONTANE ENVIRONMENTS AS A SOURCE OF BIOTYPIC DIVERSITY IN RUSSIAN WHEAT APHID, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), IN COLORADO

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), is a small grain pest of worldwide economic importance. The preferred hosts of the Russian wheat aphid are wheat (*Triticum aestivum L.*), barley (*Hordeum vulgare L.*) and triticale (X *Triticosecale* Wittmack). The aphid also colonizes noncultivated cool-season grasses, particularly wheatgrasses, and volunteer wheat and barley plants. This pest also has been found on different grass species at elevations between 1,000 and 3,000 m above sea level in Colorado.

The objective of this study was to improve our knowledge of Russian wheat aphid biotypic diversity present along the Front Range of northern Colorado. In order to achieve this objective I compared the biotypic diversity of Russian wheat aphids present on alternate grass hosts (crested wheatgrass, intermediate wheatgrass, slender wheatgrass, western wheatgrass and foxtail barley) in montane and prairie environments using plant differentials (wheat and barley lines) currently of interest to the Colorado State University wheat breeding program. The ultimate goal was to determine if noncultivated grass hosts in montane environments are a potential source of the biotypic diversity of Russian wheat aphid affecting wheat production in eastern Colorado.

Fourteen Russian wheat aphid isolates were collected in 2010 from prairie and montane sites and characterized in a standard seedling assay using 22 wheat and two barley lines with known reactions to biotype 1 Russian wheat aphid (RWA1) and biotype 2 Russian wheat aphid (RWA2). Thirty-four Russian wheat aphid isolates were collected in 2011 and characterized in a

standard seedling assay using seven wheat and one barley lines with know reactions to the RWA1 and RWA2 biotypes. Known biotypes RWA1 and RWA2 were included for comparison. Once the RWA1 susceptible wheat cultivar ‘Yuma’ and barley cultivar ‘Otis’ were killed, all plants were rated for leaf chlorosis and leaf rolling.

Differences in virulence patterns were observed among the 14 isolates from 2010 and the 34 isolates from 2011 for leaf chlorosis and leaf rolling. The isolates exhibited different levels of damage, and divided into three groups: isolates producing low amount of damage (similar to that expected by biotype RWA1 on plants expressing Dn4 resistance), isolates producing heavy damage (similar to that expected from biotype RWA2 feeding on susceptible plants or plants expressing Dn4 resistance), and isolates with intermediate level of damage.

Isolates M5 and P14, collected in 2010, produced more damage than the RWA2 biotype. Isolate M5 produced heavy damage on CO03797, Karee-Dn8, Betta-Dn9, and intermediate damage on 94M370 and Sidney, whereas RWA2 biotype produced intermediate damage on CO03797, Karee-Dn8, and Betta-Dn9, and low amount of damage on 94M370 and Sidney. Isolate P14 produced heavy damage on Karee-Dn8 and intermediate damage on 94M370, whereas RWA2 biotype produced intermediate damage on Karee-Dn8 and low amount of damage on 94M370.

Collection date and site did not influence damage in either 2010 or 2011 isolates. For example, isolates M5, M2, M3 and M4 from 2010 were all collected from the same montane site on the same date, but were categorized into three damage groups. Isolates M40, M36 and M37 were also collected from the same montane site on the same date in 2011, but represented two damage groups.

The results of these screenings confirmed the presence of two previously unknown biotypes: isolate M5, collected from a montane site, and isolate P14, collected from a prairie site. Both biotypes were more virulent than RWA2 biotype. These results also documented that biotypic diversity of Russian wheat aphid in Colorado montane was greater than the prairie collections.

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LITERATURE REVIEW

Distribution and economic importance of Russian wheat aphid

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), is a small grains pest of worldwide economic importance (Quisenberry and Peairs 1998). Since it was first detected in the United States, in 1986, it has caused crop losses of several hundred million dollars (Morrison and Peairs 1998, Souza 1998). This pest has also been found on different grass species at all elevations between 1,000 and 3,000 m in Colorado (Randolph et al. 2011).

Diuraphis noxia is indigenous to southern Russia, countries bordering the Mediterranean Sea, Iran and Afghanistan (Walters et al. 1984). Reports of mass outbreaks in Moldova and Russia are found in publications dated before 1900. Those outbreaks attracted attention because of the destructiveness of this aphid to small grains production (Poprawski et al. 1992, Miller et al. 2005). It was first recorded as a pest of wheat in South Africa during 1978 (Dürr 1983, Walters et al. 1984). Later, populations were detected in 1980 in Mexico (Gilchrist et al 1984), in the Canadian provinces of Alberta, British Columbia and Saskatchewan since 1986 (Kovalev et al. 1991), Chile in 1987 (Zerené et al. 1988), and Argentina in 1991 (Ortego y Delfino 1994). Its range also expanded in the latter part of the twentieth century to include grain production areas of Europe such as Hungary and the Czech Republic (Stary 1999, Stary et al. 2003).

Prevailing wind currents are thought to be responsible for the dispersal of Russian wheat aphid from Mexico to the United States (Stoetzel 1987). The aphid was first collected and identified in 1986 near Muleshoe, Texas, and spread rapidly throughout the western states, including: Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, Nevada, New

Mexico, North Dakota, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington, and Wyoming (Elliott et al. 1998).

In the United States, the rapid spread of *D. noxia* had the potential to devastate dryland wheat and barley production. The direct and indirect costs of Russian wheat aphid to small grain producers in the western United States totaled $893.1 million from 1987 to 1993, averaging $127 million per year (Morrison and Peairs 1998). This aphid had caused crop losses in excess of 771 metric tons of wheat, *Triticum aestivum* L. and *T. turgidum* var. *durum* L., and barley, *Hordeum vulgare* L., by 1994 (Souza 1998).

Russian wheat aphid biology

Russian wheat aphid is a relatively small (less than 2 mm long), yellow green or gray green aphid with an elongated, spindle-shaped body (Stoetzel 1987, Walters et al. 1984). The aphid has reddish eyes that protrude from the side of the head and six-segmented antennae that reach to the posterior third of the midthorax, less than half the length of the body (Dürr 1983, Miller et al. 2005, Poprawski et al. 1992). The siphunculi are short (50-60 μm long), truncate, about as long as wide, and pale in color (Dürr 1983, Stoetzel 1987). The aphid also possesses a supracaudal process, sometimes referred to as a “double tail” (Walters et al. 1984).

Russian wheat aphid exhibits both holocyclic and anholocyclic populations (Halbert and Stoetzel 1998). This pest is holocyclic in its native range (Kiriac et al. 1990), producing annual patterns of several parthenogenic generations followed by a sexual generation in the autumn that produces an overwintering egg stage. In North America, the aphid is thought to be anholocyclic with year-round parthenogenic reproduction (Burd et al. 1998). Recently, Shufran et al. (2011) mentioned that since 2003 there was an increasing occurrence of biotypes and an expansion of *D.*

noxia’s range, and also strong evidence that no additional introductions of *D. noxia* had occurred. They suggested that genetic recombination during the holocycle is likely to be a source of these biotypes. Oviparae were collected (Kiriac et al. 1990), but no males or viable overwintering eggs had been found (Halbert and Stoetzel 1998). In late March of 2007, Puterka et al. (2012) discovered a population of Russian wheat aphid fundatrices in a small patch of mixed wild grasses hosts at Dove Creek, Colorado (elevation 2,058 m). Sexual morphs or eggs were not found before the discovery of the fundatrix population. Regardless, the absence of alate adults, the infested area’s reduced size, and the presence of numerous eggs and fundatricies of *Diuraphis tritici* and *Sipha elegans* suggested that the Russian wheat aphid fundatrix population resulted from cyclical parthenogenesis.

Harsh winter conditions at high altitudes and heterogeneous environments may be some of the reasons for aphids to reproduce sexually (Frantz et al. 2006). During the fall, when temperatures decrease, sexual morphs of Aphididae usually occur to produce hardy eggs to survive the winter. Generally, holocyclic lineages occur at higher latitudes while anholocyclic lineages occur at lower latitudes or elevations. Holocyclic lineages are more common in areas with regular harsh winters because they produce cold-resistant eggs. Anholocyclic lineages inhabit mild winter areas, which favor fast asexual reproduction rates. Nevertheless, holocyclic populations can occur in temperate regions with mild winters. The possibility of inhabiting a broader geographical range or overcoming environmental variability is given by the advantage of sexual reproduction that results in genetic variability in sexual populations (Frantz et al. 2006).

Frantz et al. (2006) studied pea aphid, *Acyrthosiphon pisum* (Harris), holocycly on annual and perennial hosts. Holocycly in pea aphid was more common on annual hosts than on perennial hosts. Aphids have to colonize alternate hosts when annual crops are harvested. They explained that sexual reproduction might occur due to the selective pressure exerted by this alternate means of survival.

Host plants

The preferred hosts of the Russian wheat aphid are wheat, barley and triticale (Walters et al. 1984). The aphid also colonizes noncultivated cool-season grasses, particularly wheatgrasses, and volunteer wheat and barley (Peairs 1998).

In North America, Russian wheat aphid colonizes newly emerged cereal grains in the fall, from October to early November. Viviparous females overwinter on the crops, causing the most damage in the spring. Alate forms of the aphid appear in April and May (Peairs 1998) when the host plants are under stress or when the plants reach a growth stage that no longer provides a favorable habitat for the pest (Walters et al. 1984).

After harvest of winter grains in the summer, the aphid then moves to late maturing winter wheat, spring grains, and noncultivated hosts, such as grasses and volunteer grains (Poprawski et al. 1992, Walters et al. 1984). Apterous forms of the aphid move by crawling to neighboring plants (Poprawski et al. 1992). Alate forms fly short distances under their own power, but also can travel many kilometers using convection currents and prevailing winds (Walters et al. 1984). Russian wheat aphid oversummers on these noncultivated hosts and returns to newly emerged crops in the fall (Burd et al. 1998, Peairs 1998).

The period between harvest and planting (July-September) is when grass species, either native or introduced, are important alternate hosts of Russian wheat aphids (Armstrong et al. 1991). By mid-summer, noncultivated grasses at lower elevations typically have senesced, while grasses at higher elevations are still in vegetative and early reproductive stages (Pucherelli 2010).

Russian wheat aphid would not survive the summer and produce fall infestations without this alternate means of survival (Armstrong et al. 1991).

In a field survey of twenty-five grass species in northeastern Colorado, Russian wheat aphid was found on a wider range of hosts in the early summer in comparison to the later summer months when grasses began to senesce. Crested wheatgrass (*Agropyron cristatum*, (L.) Gaertn) and Canada wildrye (*Elymus canadensis* L.) were dominant hosts of this aphid (Armstrong et al. 1991). Weiland (2006) sampled noncultivated grass hosts in Colorado during the fall and spring of 2005, and the spring of 2006. Russian wheat aphids were collected from crested wheatgrass, downy brome (*Bromus tectorum* L.), volunteer wheat, Canada wildrye and intermediate wheatgrass (*Elytrigia intermedia*, (Host) Nevski) at most locations. Aphids were found less frequently on bottlebrush squirreltail (*Elymus multisetus*, M. E. Jones), green foxtail (*Setaria viridis*, (L.) Beauv), smooth brome (*Bromus inermis*, Leyss), and barnyard grass (*Echinochloa crusgalli*, (L.) Beauv).

Russian wheat aphid has been found on eighteen different grass species at all elevations between 1,500 and 3,000 m in Colorado. The most common hosts harboring Russian wheat aphid at high elevations were crested wheatgrass, intermediate wheatgrass (*Elytrigia intermedia*, (Host) Nevski), slender wheatgrass (*Elymus rachycaulus*, (Link) Gould ex Shinners), western wheatgrass (*Pascopyrum smithi*, (Rydb.) A. Löve), and foxtail barley (*Hordeum jubatum*, (L.) Tesky). (Randolph et al. 2011).

Plant injury

Russian wheat aphid injures the plant by inserting its stylet into the plant, and feeding from the phloem sap (Fouché et al. 1984). The salivary components elicit responses from the plant like leaf chlorosis, characterized by white, yellow or purple streaking, and leaf rolling. Russian wheat aphid feeding damage also causes ultrastructural and tissue-level damage that affects phloem composition (Cooper et al. 2010).

Common symptoms of infestation on wheat produced by Russian wheat aphid are longitudinal streaks and inward curling (rolling) on the leaves of the plant (Walters et al. 1984). Russian wheat aphid injury on wheat leads to a reduction of plant height, shoot weight, and number of spikes during tillering and jointing stages, while yield per plant is reduced at tillering, jointing and heading stages. The quality of the grain and protein content are also negatively affected by Russian wheat aphid infestations (Girma et al. 1993).

Weather conditions affecting population abundance

Russian wheat aphid population abundance varies greatly between geographic locations, and also from year to year within the same location. Favorable conditions for aphid reproduction and growth include rapid vegetative growth of acceptable host plants, and warm and dry weather, which increase the abundance of Russian wheat aphid (Hammon et al. 1997). The temperature range for Russian wheat aphids growth and development is between 5-30 ºC (Burd et al. 1998). Mortality during two critical time periods, overwintering and oversummering, is largely the reason for decline in *D. noxia* abundance (Hammon et al. 1997). The ability of the aphid to survive is closely related to temperatures fluctuations. When temperatures are low, reproduction slows and generation time increases, and when temperatures are more favorable, reproduction increases and generation time shortens. (Michels and Behle 1988). Extended cold temperatures, prolonged periods of snow cover, and several cycles of wet snow followed by rapid melt and quick freeze are detrimental to Russian wheat aphid during winter weather conditions (Hammon et al. 1997, Peairs 1998). Similarly, prolonged high temperatures are detrimental to oversummering aphids. Humidity is also an important climatic factor for *D. noxia*, though its influence it is only evident under distinct excess or deficient of moisture conditions. A sufficient amount of humidity makes the epidermal layers of plants soft and easily pierced by the aphid. Drought conditions make plants difficult to feed on because the epidermal layers of plants are thick and hard. Too much humidity can result in a fungal infection of the aphid (Poprawski et al. 1992). The amount of precipitation and/or irrigation is another important host plant factor. Plants that are infested with *D. noxia* under drought conditions can suffer from significant damage (Johnson et al. 1998). Another physical factors affecting Russian wheat aphid abundance is wind, important to migration (Poprawski et al. 1992)

Management tactics for Russian wheat aphid

Monitoring

Russian wheat aphid is an annual threat to small grain productions in the western United States (Hammon et al. 1997). Monitoring of Russian wheat aphid populations is a practice used to determine the level of infestation and to help planning for future outbreaks in a certain area (Weiland 2006). Sweep net sampling and visual inspection of tillers and plants are some useful techniques used for monitoring aphids (Archer and Bynum 1992, Stary 1999). A two-field study conducted by Archer and Bynum (1992, 1993) was used to evaluate two sampling units, whole plants and individual tillers, for determining the infestation of Russian wheat aphid in dryland winter wheat. In those studies, they concluded that either whole plants or tillers can be used as the sample unit to estimate aphid numbers, but the most accurate was infested tillers because it has a stronger correlation with yield, and was more time efficient.

Another implement used to monitor Russian wheat aphid flights in Colorado is the Allison-Pike suction trap. The trap consists of a 12-inch diameter tube that stands 26 feet above the ground (Hammon et al. 1997), which captures aphids that fly over the tube. Then, the aphids are collected and counted in order to estimate local aphid abundance (Weiland 2006). Suction traps show the presence of Russian wheat aphid in an area of approximately 30 km radius from the trap (Halbert et al. 1990). Although suction traps are important in determining aphid movements and levels, Stary and Lukasova (2000) recommended also sampling for Russian wheat aphid directly from the crop, at the early ripening stage, because the samples collected by the suction trap only corresponds with alate aphids dispersing from diminishing populations.

Biological Control

Pesticide applications increase the direct costs for controlling Russian wheat aphid. This practice is considered to be one of the reasons for the high total losses produced by this pest (Wraight et al. 1993). The use of biological control agents, as predators, parasites, and pathogens, was evaluated in Colorado. In June 1990, Wraight et al. (1993) conducted field surveys to accumulate data about existing natural enemies of Russian wheat aphid. Parasite occurrence was low (<5%), with *Diaeretiella rapae* (M'Intoch) being the most common parasitoid. The only predators found feeding on *D. noxia* within rolled leaves were syrphid larvaes, but the populations were low, < 0.3 larvae per aphid-infested tiller. Three species of pathogenic fungi were found, but their incidence was less than 2.5%, suggesting that irrigation is required for this biological control agent to be an effective means of control. This was confirmed by Knudsen and Wang (1998) who showed that *D. noxia* populations were reduced in controlled environment studies by the entomopathogenic fungus, *Beauveria bassiana*, but not in field treatments.

From 1991 through 1993, seven exotic species of hymenopterous parasitoids (*Aphelinus asychis* (Walker), *Aphelinus albipodus* (Hayat and Fatima), *Aphelinus varipes* (Forester), *Diaeretiella rapae* (M'Intosh), *Aphidius colemani* (Viereck), *Aphidius matricariae* (Haliday), and *Ephedrus plagiator* (Nees), were released at several sites in eastern Colorado. One year later, in 1994, the recovery of *A. asychis*, *A. albipodus* and *D. rapae* indicated that these species have been established, though *A. asychis* and *D. rapae* were already known from the state, so it is difficult to know whether the released populations of these two species established (Elliott et al. 1995, Burd et al. 2001). From 1991 to 1994, Mohammed at al. (2000) collected 41 natural enemies of *D. noxia* on an organic farm in Colorado. Most common were coccinellids and nabids. *D. rapae* was the only primary parasitoid collected in the study. A high number of hyperparasitoids were found associated with *D. rapae*. This could be the reason for the low population and efficacy of *D. rapae* against *D. noxia*. In the same study, in April- May 1992, these authors also released four predatory species (*Eupeodes nuda* (F.), *Hippodamia variegata* (Goeze), *Leucopis ninae* Tanasijtshuk, and *Propylea quatuordecimpunctata* (L.) and four parasitoids species (*A. asychis*, *A. varipes*, *A. matricariae*, and *D. rapae*). At the end of the study in 1994, only one individual of *P. quatuordecimpunctata*, a small colony of *L. ninae* Tanasijtshuk and two species of parasitoids, *A. asychis* and *D. rapae*, were found, and *D. rapae* was already established from previous introductions. Larger releases of these species and a suitable environment conditions are likely necessary to have a successful establishment of these biological control agents.

Noma et al. (2005) sampled from April through October in 2001 and 2002 in southeastern Wyoming, western Nebraska, and north-central Colorado for parasitoids and predatory flies which had been released between 5-6 years prior. They detected (in order of high and low density across the sampled states, and during the 2 years of study): *A. albipodus, Eupeodes volucris* Osten Sacken (Diptera: Syrphidae), *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae), *Leucopis gaimarii* Tanasijtshuk (Diptera: Chamaemyiidae), *Aphidius avenaphis* (Fitch), *A. matricariae, D. rapae, Aphidius ervi* Haliday, *Praon yakimanum* Pike and Stary (Hymenoptera: Braconidae, Aphidiinae), and *A. asychis*. They concluded that *A. albipodus* had successfully established in the sampled area, and that was uncertain if *A. asychis*, *A. matricariae,* and *D. rapae* detections were due to exotic introductions or preexisting populations.

Randolph et al. (2002) evaluated augmentative releases of the generalist predators *Hippodamia convergens* Guerin –Meneville and *Chrysoperla rufilabris* Stephans, in 1994, 1995 and 1997. They used two treatments, total exclusion with cages and no exclusion, and measured number of Russian wheat aphid per tiller, percentage of infested tillers, and crop biomass production. There were no effects of the treatments on the measured parameters. They concluded that the commercial release of these biological control agents was not economically beneficial, because they didn’t observe any reduction in Russian wheat aphid population densities nor protection of crop biomass due to the use of these agents.

In 2007, 2008 and 2009, Pucherelli (2010) conducted a survey to document the presence of aphid predators at high elevations in Colorado. Spiders were the most abundant predators collected, with Philodromidae, Lycosidae, and Linyphiidae being the most common families. Harvestmen were also commonly found in the survey, but only the family Phalangiidae was collected. Also, eleven insect predator families were collected, most commonly Lygaeidae (subfamily: Geocorinae), Carabidae, Coccinellidae, Nabidae, and Staphyinidae. Parasitoids were not sampled because aphid populations were not large enough to collect and place in emergence canisters, although parasitized Russian wheat aphids were observed at site 10a.

Cultural Control

Cultural practices are significantly important to control insect pest of low-return extensive crops, like wheat and barley, because profit margins are not high enough to consider using insecticides. Modifications in some cultural practices, for example sanitation, grazing, fertilization, irrigation, row spacing, and planting date, can reduce infestation levels of Russian wheat aphids in small grains (Peairs 1998b).

Planting date modification is one of the most common methods used to reduce Russian wheat aphid infestations. Small grains can be planted within an agronomically acceptable range of dates without compromising yield and quality of the crop. Spring crops are recommended to be planted as early as possible, because more mature plants are less attractive to Russian wheat aphid. In the case of fall crops, planting date varies according to the site. For places where spring infestations are predominant, early fall seeding is recommended, because plants are going to be more mature and less attractive for the aphids (Peairs 1998, Peairs 1998b, Walters et al. 1984). In places where fall infestations are the major concern, fall crops should be planted as late as possible, so that the plants are smaller and less attractive for the aphids or even might emerge after aphids are gone (Hammon et al. 1996, Peairs 1998b, Walters et al. 1984).

Volunteer wheat is the most important source of infestation for the new crop in the fall. Control practices like sanitation, which consists in removing volunteer crop plants as well as crops residues, weeds and alternate hosts, grazing and tillage are used to suppress the incidence of this infestation source (Peairs 1998b, Poprawski et al. 1992, Walters et al. 1984). Pastures, which are planted earlier than cereal crops, should not include wheat, barley, or triticale, because they can provide refuge for Russian wheat aphid (Walters et al. 1984).

The use of locally adapted seeds, proper fertilization, dense crops, and optimal moisture conditions results in healthy and vigorous plants that are more resistant to damage from Russian wheat aphids and other sources of stress (Peairs 1998, Peairs 1998b, Walters et al. 1984).

Chemical Control

One year after the introduction of Russian wheat aphid in South Africa, it became noticeable that the insecticides that had been used to control Russian wheat aphid were becoming ineffective. Therefore, to obtain better results, contact and systemic insecticides were combined to control this pest (Walters et al. 1984). For preventive treatments, granular formulations of disulfoton and phorate were recommended for soil applications at planting time. Chlorpyrifos and other mixtures of systemic and contact insecticides were recommended in both ground and aerial spraying as corrective treatments, with both application methods being equally effective (Botha 1984, Walters et al. 1984).

In South Africa, Du Toit (1984) and Butts and Walters (1984) conducted experiments to test preventative insecticides to control Russian wheat aphid. Du Toit (1984) tested the efficacy of granular formulations of phorate and disulfoton applied as planting furrow treatments. Both insecticides suppressed the progress of Russian wheat aphid infestation during the winter, resulting in low spring infestations and increasing yields. Butts and Walters (1984) tested several dosages of eleven systemic insecticides as seed treatments, measuring the effects on seed germination, seedling emergence, and aphid control. Seven insecticides showed phytotoxic effects on seed germination, and three insecticides decreased seedling emergence, and they were discarded. CGA 73102 40 WP was the only insecticide that did not affect germination nor seeding emergence, and thus would be considered a favorable control for *D. noxia*.

Insecticides used in integrated pest management programs should be selective against the pest, and have little or no effect on natural enemy populations. In Texas, Bayoun et al. (1995) conducted laboratory bioassays to identify insecticide toxicity levels on Russian wheat aphid, and related parasites and predators. From the insecticides tested, chlorpyrifos, dimethoate, acephate and esfenvalerate were selectively toxic to *D. noxia*. Acephate was the least toxic insecticide for both parasites and predators, and also showed high systemic toxicity to *D. noxia*, providing a good choice for aphid management programs. However, it is not approved for use in wheat.

Chemical control is still considered an effective way to control Russian wheat aphid. For an effective control, it is important to use proper application methods, including correct dosage of insecticides, adequate spraying apparatus and exact calibration of the nozzles (Peairs 1998, Walters et al. 1984). Some insecticides currently labeled for controlling Russian wheat aphid are imidacloprid, chlorpyrifos, cyfluthrin, thiamethoxam, and dimethoate (Peairs 2006). In order to determine the need of insecticide treatments and its economic viability, it is important to inspect the fields regularly and use economic thresholds (Peairs 1998, Walters et al. 1984).

Integrated pest management tactics require the use of economic injury levels and thresholds to determine infestation levels of Russian wheat aphid. Without using an economic threshold, a pest could be sprayed with insecticides regardless of whether its damage potential is real or not (Legg and Archer 1998). Du Toit (1986) calculated the first economic threshold for Russian wheat aphid in South Africa using as the determining factor the percentage of infested plants at several growth stages (Archer et al. 1998, Legg and Archer 1998). In the United States, Archer et al. (1998) conducted research in four states (Colorado, Montana, Texas and Washington) to determine the economic injury level for Russian wheat aphid, accounting the effects of climate zone and wheat growth stage. Losses from Russian wheat aphid infestations during the spring were similar in all states, but losses due to fall infestations varied by climate zone. Different fall infestation economic injury levels, thus, would have to be used in different climate zones in the United States.

The economic threshold for Russian wheat aphid in the western Great Plains can be calculated with the following formula proposed by Peairs (1998):

$ET = (CC*200) / (EY*MV)$

Where: ET = Economic threshold, CC = control cost per acre, EY = expected yield, and MV = market value per bushel. The 200 in the numerator of the formula is substituted for 500 after flowering (Archer et al. 1998, Peairs 1998). A treatment should be cost effective if the calculated ET is lower than the percent of infested tillers observed (Peairs 1998).

Yield-infestation relationships obtained from field experiments are the basis for economic thresholds, but these relationships usually vary temporally and spatially, tend to be site specific, very expensive and time consuming. Chander et al. (2006) used a generic crop growth model, Infocrop, to simulate the damage produced by Russian wheat aphid on winter wheat in Colorado, and to determine economic thresholds through this simulation model. They found a high correlation between the observed and the simulated yield reductions in the experiments. The economic injury levels for Russian wheat aphid determined with the simulation model showed that winter wheat was more likely to be attacked during early growth stages than during later stages. There was a direct relationship between the cost of control and the change of the economic injury level through the years, and an inverse relationship with the market value of winter wheat.

Plant Resistance

Plant resistance is an economically feasible and effective method for controlling Russian wheat aphid. There are three general mechanisms of resistance describing the relationship between the insect and the host plant: antixenosis (or non-preference) in which colonization is reduced; antibiosis, in which host plants produce a negative effect on insect growth, reproduction or survival, and tolerance, in which host plants that can support a similar population as found on susceptible plants without affecting their growth rate and reproduction (Auclair 1989). Once *D. noxia* became a pest in the United States, Webster et al. (1987) tested germplasm resistant to greenbug, *Schizaphis graminum* (Rondani), for potential use in a Russian wheat aphid resistance program. They found high levels of antibiosis, antixenosis and tolerance to *D. noxia* in ‘Nora’ oats, and ‘Elbon’ rye, but the tested wheat and barley lines were susceptible to *D. noxia*. The Western Regional Coordinating Committee No. 66 (WRCC-66) assisted the development and identification of resistant sources in cultivated cereal plants (Souza 1998). They evaluated more than 25,000 lines of wheat, barley, and other cereal species, and found that the most common sources of resistance expressed combinations of antibiosis and tolerance. They also documented six genes (*Dn1*, *Dn2*, *dn3*, *Dn4*, *Dn5,* and *Dn6*) that conferred resistance in wheat, two genes in barley, and 1 gene in triticale. Many improved lines of wheat and barley were selected and released by the participating programs of WRCC-66. In 1994, Colorado released the resistant wheat cultivar ‘Halt’, which carries the resistant gene *Dn4* (Quick 1996). Hawley et al. (2003)

compared the resistant hard red wheat Halt with the susceptible wheat ‘TAM 107’ to determine the level of resistance to the Russian wheat aphid. They found antixenosis and antibiosis expressed in Halt at growing stage Zadoks 30 and 40, respectively. They also found a significant expression of tolerance, which could make Halt a good choice for integrated pest management programs. Randolph et al. (2003) determined plant damage and yield on susceptible winter wheat TAM 107 and resistant winter wheat RWA E1. They found higher infestations of Russian wheat aphid on TAM 107, and, consequently, greater yield losses.

In order to examine categories of resistance expressed in resistance sources used in Colorado, Miller et al. (2003) evaluated three winter wheats (PI 372129, PI 243781, and PI 222668), and found different levels of antixenosis, antibiosis and tolerance among the three lines. Randolph et al. (2005a) conducted a field experiment to determine if *Dn4* gene is affected by genetic background. They also found different levels of antixenosis, antibiosis and tolerance in four *Dn4* resistant winter wheat cultivars (‘Prairie Red’, Halt, ‘Prowers 99’, and ‘Yumar’). They concluded that some cultivars containing the *Dn4* gene might express some categories of resistance, while others may not show those categories. Therefore, the expression of resistance was affected by genetic background.

It is beneficial to plant resistant wheat in areas where there is a great chance for Russian wheat aphid infestations, even though yield varies among sites and resistant sources (Randolph et al. 2005b). By 2006, the available resistant varieties of wheat in Colorado included ‘Ankor’, ‘Bond CL’, Halt, ‘Hatcher’, Prairie Red, Prowers 99, ‘Ripper’, ‘Stanton’, and Yumar (Peairs 2006, Weiland 2006, Haley et al. 2007). ‘Bill Brown’ and ‘Thunder CL’ were released in August 2007 and 2008, respectively (Haley et al. 2008, Haley et al. 2009).These resistance sources were effective against the original biotype of the Russian wheat aphid. In 2003, a more virulent biotype 2 (RWA2) was identified in Colorado; thus, all these resistant varieties of wheat became ineffective against this new biotype (Haley et al. 2004). In a greenhouse seedling screening tests with resistant and susceptible cultivars, Haley et al. (2004) found that only one line, 94M370 (*Dn7*) showed resistance to this new biotype. Unfortunately, the Dn7 resistance gene is carried on the 1BL.1RS wheat-rye translocation, which is associated with poor baking quality.

After the discovery of RWA2, efforts were directed to obtain new lines of wheat and barley resistant to the biotype. From a collection of 761 wheat germplasm accessions, Collins et al. (2005) identified 44 germplasm accessions that had high to moderate levels of resistance to RWA2. Ten accessions had a high level of resistance, similar to 94M370; ‘CI 2401’ was one of these accessions. They concluded that these ten accessions should be considered in future genetic studies and breeding programs for resistance to RWA2. Qureshi et al. (2006) compared, in a greenhouse with controlled light and temperature, the development and reproduction of RWA2 on a susceptible commercial cultivar, ‘Trego’ (PI 612576), and on the lines CI 2401, a pure wheat line from Tajikistan, and ‘03GD1378027’, a USDA-ARS breeding line originated from crosses with a South African line carrying a rye translocation, which confers resistance to *D. noxia*. Lines CI 2401 and 03GD1378027 negatively affected the development and reproduction of biotype RWA2. They concluded that these lines have the potential to be effective sources of resistance against this biotype. Voothuluru et al. (2006) categorized the resistance in CI 2401 to RWA2. The rate of reproduction of the aphid was drastically reduced by the strong antibiosis effect of CI 2401. Antixenosis was not detected, and CI 2401 plants showed tolerance to leaf rolling and chlorosis.

Bregitzer et al. (2008) registered ‘RWA 1758’, a Russian wheat aphid- resistant spring barley, while Mornhinweg et al. (2008) registered seven spring two-rowed barley lines resistant to Russian wheat aphid: 'STARS 0637B' (PI 642923), 'STARS 0638B' (PI 642924), 'STARS 0639B' (PI 642925), 'STARS 0640B' (PI 642926), 'STARS 0641B' (PI 642927), 'STARS 0642B' (PI 642928), and 'STARS 0643B' (PI 642929).

Lazzari et al. (2009) evaluated wheat lines containing the resistant genes *Dnx*, *Dn7*, *Dn6* and *Dn4*, resistant to the Russian wheat aphid, along with a susceptible control, to determine the categories of *D. noxia* biotype 2 (RWA2) resistances for each genotype, and feeding behavior on *Dnx* and the susceptible control. They found that plants with *Dnx* resistant gene showed antibiosis resistance to RWA2 similar to those containing Dn7 gene. The advantage of *Dnx* resistant gene is that it is derived from hexaploid wheat, instead of the rye-based Dn7 gene, that has negative quality baking traits.

From 2009 to 2012, more resistant lines to Russian wheat aphid have been registered in the United States: 'Sidney' spring feed barley (Mornhinweg et al. 2009), six wheat-rye addition lines 'CO03752' (PI 659317); 'CO03754' (PI 659318); 'CO03758' (PI 659319); 'CO03761' (PI 659320); 'CO03764' (PI 659321); and 'CO03765' (PI 659322) (Nkongolo et al. 2011), Stoneham spring feed barley (Mornhinweg et al. 2012a), and eight six-rowed feed barley lines resistant to both Russian wheat aphid and greenbug 'STARS 1006B' (PI 659760), 'STARS 1007B' (PI 659761), 'STARS 1008B' (PI 659762), 'STARS 1009B' (PI 659763), 'STARS 1010B' (PI 659764), 'STARS 1011B' (PI 659765), 'STARS 1012B' (PI 659766), and 'STARS 1013B' (PI 659767) (Mornhinweg et al. 2012b).

It's important to identify the molecular basis of genetic resistant lines to understand the mechanisms of *D. noxia* resistance. One strategy to diminish the selection for virulence in the aphid population is to incorporate in commercial wheat cultivars a more diverse pool of genes resistant to *D. noxia* (Qureshi et al. 2006). This has been the direction followed by small grains breeding programs to find resistant lines to RWA2.

Russian wheat aphid biotypes

An insect biotype is a population of insects that are able to damage host plants that were previously resistant to that specific insect species (Puterka et al. 1988). Russian wheat aphids have been monitored for biotypic diversity, because new biotypes can potentially disrupt the progress of plant breeding programs that have developed resistance. Biotypes are determined by exposing aphid populations to plant differentials (wheat, rye, and barley cultivars). Plant differentials are sets of plant cultivars used to define biotypes of insects based on known susceptible and resistant reactions. Aphids that are avirulent to specific cultivars will not damage those plants. New biotypes are described when aphid populations damage plants previously known to be resistant (Pucherelli 2010).

In North America, only one Russian wheat aphid biotype (designated as RWA1) was known prior to the spring of 2003 when a new biotype was discovered in southeastern Colorado (designated as RWA2) causing damage on the cultivar Prairie Red, carrying the *Dn4* resistance gene (Haley et al. 2004). Since then, several other biotypes have been described in North America. Burd et al. (2006) conducted a survey from May through June 2002 and August 2003 in Kansas, Nebraska, Texas and Wyoming to determine Russian wheat aphid biotypic diversity. They found three new Russian wheat aphid biotypes (RWA3, RWA4, and RWA5) in cultivated wheat and barley. In Colorado, Weiland et al. (2008) obtained an isolate from aphids collected in Montezuma County in 2004. The isolate presented a unique virulence profile and was designated as RWA6. In 2005, they collected biotype RWA7 from Canada wildrye and intermediate wheatgrass and RWA8 from crested wheat grass and smooth brome.

One hypothesis for Russian wheat aphid biotypic diversity in North America is related to holocycly. Through sexual selection, Russian wheat aphid populations may evolve to different biotypes. This hypothesis is supported by the discovery of oviparae in North America (Kiriac et al. 1990). The selective pressure produced by resistant cultivars and alternate host could also result in more virulent biotypes of Russian wheat aphid (Merrill et al. 2008). While studying greenbug biotype development, Porter et al. (1997) suggested that noncultivated grass hosts could be a reservoir for aphid biotypes. Since then, several unique greenbug biotypes have been found on noncultivated hosts (Anstead et al. 2003, Burd and Porter 2006). Regarding the effect of noncultivated grasses on Russian wheat aphid biotipic diversity, the two biotypes RWA6 and RWA7 found by Weiland et al. (2008) on noncultivated grasses, on Colorado plain locations, were later confirmed as new biotypes by Randolph et al. (2009).

INTRODUCTION

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), is a small grain pest of worldwide economic importance (Quisenberry and Peairs 1998). Since it was first detected in the United States in 1986, it has caused crop losses of several hundred million dollars (Morrison and Peairs 1998, Souza 1998). The preferred hosts of the Russian wheat aphid are wheat (*Triticum aestivum L.*), barley (*Hordeum vulgare L.*) and triticale (X *Triticosecale* Wittmack) (Walters et al. 1984). The aphid also colonizes noncultivated cool-season grasses, particularly wheatgrasses, and wheat and barley volunteers (Peairs 1998). This pest has also been found on different grass species between 1,000 and 3,000 m in Colorado (Randolph et al. 2011). The most common hosts harboring Russian wheat aphid at high elevations are crested wheatgrass (*Agropyron cristatum*, (L.) Gaertn), intermediate wheatgrass (*Elytrigia intermedia*, (Host) Nevski), slender wheatgrass (*Elymus rachycaulus*, (Link) Gould ex Shinners), western wheatgrass (*Pascopyrum smithi*, (Rydb.) A. Löve), and foxtail barley (*Hordeum jubatum*, (L.) Tesky). (Randolph et al. 2011).

The time period between harvest and planting (July-September) is when grass species, either native or introduced are important alternate hosts of Russian wheat aphids (Armstrong et al. 1991). By mid-summer, noncultivated grasses at lower elevations typically have senesced, while grasses at higher elevations are still in vegetative and early reproductive stages (Pucherelli 2010). Russian wheat aphid would not survive the summer and produce fall infestations without this alternate means of survival (Armstrong et al. 1991).

In North America, only one Russian wheat aphid biotype (designated as RWA1) was known prior to the spring of 2003 when a new biotype was discovered in southeastern Colorado, designated as RWA2 (Haley et al. 2004). Several other biotypes have since been described. Burd et al. (2006) surveyed Kansas, Nebraska, Texas, and Wyoming for Russian wheat aphid biotypic diversity, and described three new Russian wheat aphid biotypes (RWA3, RWA4, and RWA5) from cultivated wheat and barley. An isolate, that is, an isofemale colony generated from a single aphid, from Montezuma County, Colorado, from cultivated wheat had a unique virulence profile and was designated as RWA6. The intermediate level of virulence caused by Russian wheat aphid shows the importance of noncultivated grasses. Biotype RWA7 was collected from Canada wildrye and intermediate wheatgrass, and biotype RWA8 from crested wheatgrass and smooth brome (Weiland et al. 2008).

One hypothesis for Russian wheat aphid biotypic diversity in North America involves sexual selection resulting from holocycly. Support for this hypothesis comes from the discovery of oviparae in North America (Kiriac et al. 1990). Biotype diversity could also result from selective pressures exerted by resistant cultivars (Merrill et al. 2008). Porter et al. (1997) studied greenbug biotype development and suggested that noncultivated grass hosts could be a reservoir for aphid biotypes. Since this theory was suggested several unique greenbug biotypes have been found on noncultivated hosts (Anstead et al. 2003, Burd and Porter 2006). Similarly, Weiland et al. (2008) found two new Russian wheat aphid biotypes on noncultivated grasses, at locations on the Colorado plains.

Based on the following knowledge: (1) the identification and occurrence of Russian wheat aphid on noncultivated grass hosts in mountain environments (Pucherelli 2010), (2) the determination of new biotypes of Russian wheat aphid on noncultivated grass hosts in prairie environments (Weiland et al. 2008), and (3) holocycly is being more likely in the harsher (especially winter) environments found at higher elevations (Pucherelli 2010, Frantz et al. 2006), it is important to determine the diversity of Russian wheat aphid biotypes present on noncultivated grass hosts in montane environments, in order to evaluate the potential of this environment as possible source of new biotypes.

The objective of this study was to improve our knowledge of Russian wheat aphid biotypic diversity present along the Front Range of northern Colorado. In order to achieve this objective I compared the biotypic diversity of Russian wheat aphid present on alternate grass hosts (crested wheatgrass, intermediate wheatgrass, slender wheatgrass, western wheatgrass and foxtail barley) in montane and prairie environments using plant differentials (wheat and barley lines) currently of interest to the Colorado State University wheat breeding program. The ultimate goal was to determine if noncultivated grass hosts in montane environments are a potential source of the biotypic diversity of Russian wheat aphid affecting wheat production in eastern Colorado.

MATERIALS AND METHODS

I. Noncultivated host sites

Montane environments

Each of the 13 sites (Figure 1) was sampled every two weeks from the beginning of July to the end of October of 2010 and 2011. Sites were selected along Colorado Highway 14 through the Cache La Poudre River Canyon, west of Fort Collins, in Larimer County, Colorado. All sites were located along an elevation gradient ranging from 1,891 to 2,698 m on Roosevelt National Forest land. Sites were selected on the basis of accessibility and because they occurred at elevations higher than eastern Colorado wheat production. GPS data were recorded for each location. The selected sites were a subset of those sampled by Pucherelli (2010) (Table 1 and Figure 2).

Grass species sampled were: crested wheatgrass, intermediate wheatgrass, slender wheatgrass, western wheatgrass, and foxtail barley. These are known to be suitable Russian wheat aphid hosts (Pucherelli 2010).

Prairie environments

Grass samples were collected during the same periods as described for montane environments. The seven sites were located in Weld County near Briggsdale and Raymer, in Logan County near Sterling, in Washington County near Akron, and in the north limit of Morgan County, CO (Figure 1). One site had been sampled previously by Weiland (2006) while the remainder of the sites were new. The grasses sampled were the same as those listed from

Pucherelli (2010), with the exception of foxtail barley, which was not found at the prairie sites (Table 1 and Figure 3).

II. Aphid Collection Methods

Two or three handfuls of grass (approximately 30 to 40 stems) from each site were cut at ground level and placed in a pre-labeled plastic bag (Figure 4). Grass samples were stored in a cooler with ice packs to keep aphids alive during transport to the Colorado State University Agricultural Research and Development Center (ARDEC), Larimer County, Colorado, where they were placed in 20 l sheet metal Berlese funnels for approximately 12 hours to extract live aphids into cups containing leaf cuttings from wheat seedlings. A small piece of moist paper towel was also placed in each cup to prevent aphid desiccation.

All live female Russian wheat aphids collected in this manner were transferred to individual clip cages placed on the leaf of a wheat or barley plant from a pot containing a mixture of wheat and barley and covered with organza to prevent cross contamination between pots (Figures 5 and 6). The objective of this procedure was to begin an isofemale colony from that single aphid. Once the single female aphid had produced 8 or 9 nymphs, the clip cage was removed and the aphids were maintained in that same pot. Isolates were maintained in the greenhouse until a sufficient number of aphids were available for the biotype assay.

Figure 1: Location of sites sampled for Russian wheat aphid biotypes on noncultivated hosts in 2010 and 2011 in Logan, Washington, Weld and Larimer counties, Colorado

Legend:

📌 Montane sites

📌 Prairie sites

— CO Hwy 14

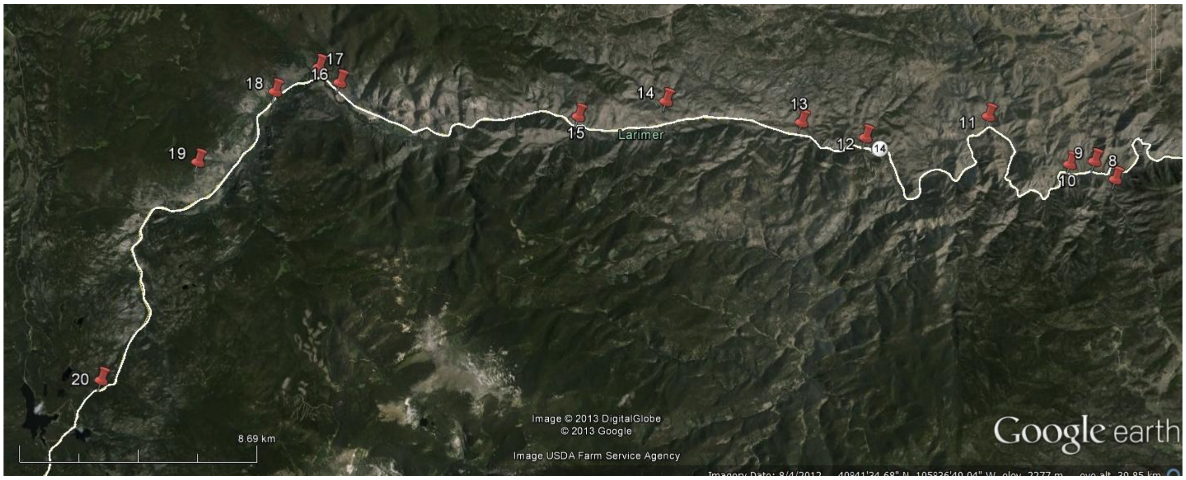

Figure 2: Montane sites sampled for Russian wheat aphid biotypes on noncultivated hosts in 2010 and 2011 in Larimer county, Colorado

Legend:

📌 Montane sites

▬ CO Hwy 14

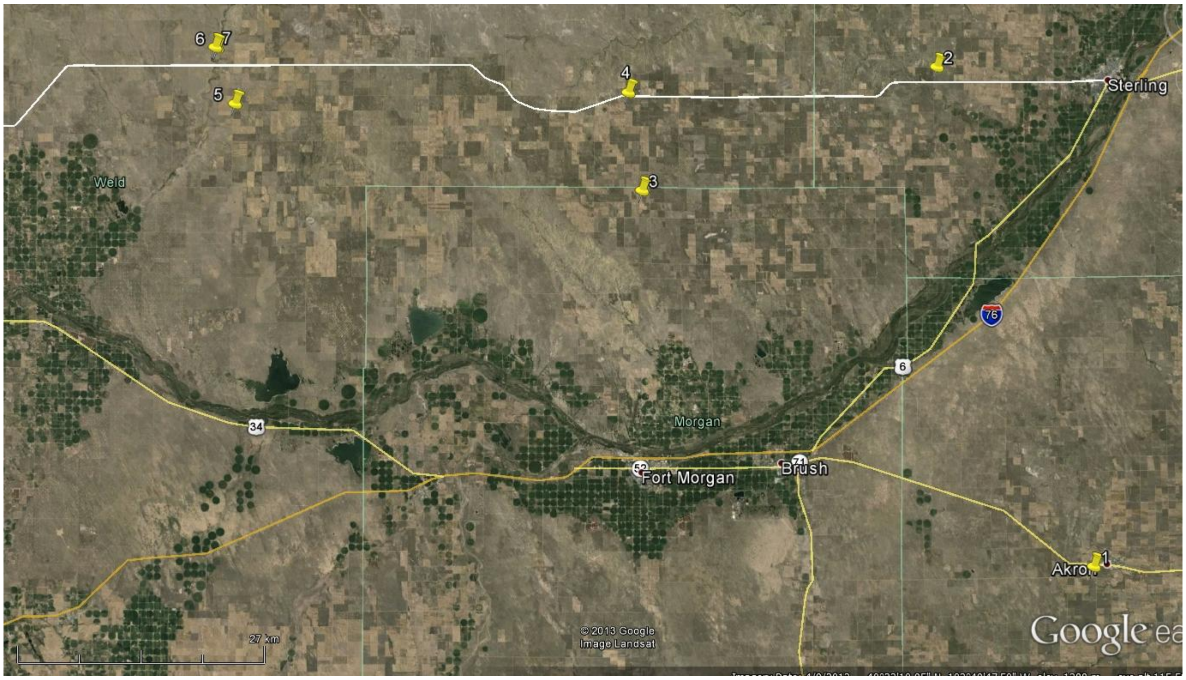

Figure 3: Prairie sites sampled for Russian wheat aphid biotypes on noncultivated hosts in 2010 and 2011 in Logan, Washington, and Weld counties, Colorado

Legend:

📌 Prairie sites

▬ CO Hwy 14

Table 1: Geographic location, noncultivated hosts sampled, and sample history for the 20 northeastern Colorado sites sampled in 2010 and 2011

Site #	**Grass sampled**	**Latitude and longitude**	**Location**	**Corresponds to new site, or Weiland (2006), or Pucherelli (2010) [a] # sites**
1	Intermediate	40.147 N, 103.235 W	Prairie	New site (Washington County)
2	Intermediate	40.628 N, 103.430 W		New site (Logan County)
3	Crested	40.510 N, 103.804 W		New site (Morgan County)
4	Intermediate	40.604 N, 103.821 W		New site (Weld County)
5	Crested	40.595 N, 104.318W		1, Weld County, Weiland
6	Crested	40.645 N, 104.342W		New site (Weld County)
7	Western	40.646 N, 104.344 W		New site (Weld County)
8	Slender	40.677 N, 105.388 W	Montane	1, Pucherelli
9	Crested	40.683 N, 105.397 W		3, Pucherelli
10	Western	40.682 N, 105.408 W		4a, Pucherelli
11	Crested	40.698 N, 105.444 W		6', Pucherelli [b]
12	Crested	40.691 N, 105.498 W		7, Pucherelli
13	Crested	40.696 N, 105.526 W		8a, Pucherelli
14	Intermediate	40.703 N, 105.585 W		10a, Pucherelli
15	Crested	40.698 N, 105.623 W		12a, Pucherelli
16	Crested	40.709 N, 105.726 W		14, Pucherelli
17	Intermediate	40.714 N, 105.735 W		15, Pucherelli
18	Crested	40.706 N, 105.754 W		16, Pucherelli
19	Crested	40.683 N, 105.785 W		17, Pucherelli
20	Foxtail	40.612 N, 105.827 W		22b, Pucherelli

[a] Pucherelli sites are all located in Larimer County.

[b] Corresponds to the suction trap site used by Pucherelli (2010) at Dutch George, mile marker 101, Roosevelt National Forest, Larimer County, Colorado.

Figure 4: Sampling noncultivated grasses at a montane site in 2010

Figure 5: Clip cages in which individual Russian wheat aphids were placed in order to start isofemale lines

III. Initial biotype screening

Once isolates had increased sufficiently (n ~1000), aphids from each colony were placed on a new pot containing three wheat differentials, ‘Yuma’ (PI 559720, susceptible check), ‘Yumar’ (PI 605388, *Dn4*) and ‘CO08RWA50’ (PI 664301, *Dn7*). Differentials were evaluated when the susceptible check, Yuma, was dead. Biotypic status of each sample was determined based on known differential responses. RWA1 killed only the susceptible Yuma, showing no symptoms on the other differentials. Isolates showing virulence to Yumar or CO08RWA50 were designated ‘not RWA1 biotype’ and subsequently biotyped on a full set of wheat and barley differentials (Table 4).

Twenty-three isolates were established in 2010, four from prairie sites, and 19 from montane sites. The four isolates from prairie sites were all ‘not RWA1 biotype’ based on the initial screening. The 19 isolates from montane sites were nine RWA1 biotype (47.4% RWA1), and ten ‘not RWA1 biotype’ (52.6% ‘not RWA1 biotype’) based on the initial screening. The nine RWA1 biotype isolates were from site 9 (one isolate collected on 12 August 2010, and 3 isolates collected on 26 August 2010), site 11 (four isolates collected on 26 August 2010) and site 19 (one isolate collected on 26 August 2010) from montane locations (Table 2). The four ‘not RWA1 biotype’ isolates from prairie sites and the ten ‘not RWA1 biotype’ isolates from montane sites were maintained in the greenhouse for future biotyping against a full set of differentials.

One hundred isolates were established in 2011, 29 from prairie sites, and 71 from montane sites. The 29 isolates from prairie sites were one RWA1 biotype (3.4% RWA1 biotype), and 28 ‘not RWA1 biotype’ (96.6% ‘not RWA1 biotype’) based on the initial screening. The only RWA1 biotype isolate was from site 3, collected on 28 July 2011, from a prairie location.

The 71 isolates from montane sites were 12 RWA1 biotype (16.9% RWA1 biotype), and 59 'not RWA1 biotype' (83.1% 'not RWA1 biotype') based on the initial screening. The 12 RWA1 biotype isolates were from site 9 (one isolate collected on 7 July 2011, one isolate collected on 21 July 2011, two isolates collected on 04 August 2011, and one isolate collected on 19 August 2011), site 11 (one isolate collected on 21 July 2011, and two isolates collected on 19 August 2011), site 13 (two isolates collected on 021 July 2011), site 18 (one isolate collected on 22 September 2011), and site 14 (one isolate collected on 6 October 2011) (Table 3).

By January 2012, a total of seven isolates from 2011 were lost due to an infestation of parasitoids in the greenhouse, two were from prairie sites, and five were from montane sites. Consequently, 26 'not RWA1 biotype' isolates from prairie sites and 54 'not RWA1 biotype' isolates from montane sites were maintained in the greenhouse for future biotyping against a full set of differentials.

IV. Determination of Biotypes

Fourteen and 80 isofemale lines, established from the isolates collected in 2010 and 2011, respectively, were maintained in the greenhouse for biotyping against a full set of differentials (Tables 2 and 3). Due to the lack of time and space in the growth room, only the results for 34 isolates from 2011 are presented here.

The 14 isolates from 2010 were biotyped using 24 wheat and barley lines with known reactions to RWA1 and RWA2 (Table 4). Known biotypes RWA1 and RWA2 were included in each screening for comparison.

Table 2: Collection dates and locations for 14 Russian wheat aphid isolates collected from noncultivated grasses in northeastern Colorado in 2010 characterized for biotypic diversity.

Colony number[a]	Sampling date	Site[b]
M2	08/12/2010	11 a
M3	08/12/2010	11 b
M4	08/12/2010	11 c
M5	08/12/2010	11 d
P6	08/18/2010	6 b
P7	08/18/2010	6 c
M8	08/26/2010	9 a
M9	08/26/2010	9 f
M10	08/26/2010	11 a
M11	08/26/2010	11 e
M12	08/26/2010	11 f
M13	08/26/2010	11 h
P14	09/02/2010	6 a
P15	09/02/2010	6 b

[a] the upper case letter before the colony number indicates from where it was collected, M= Montane site, P= Prairie site.
[b] the letter after the site number identifies each different colony established at the same site on the same date.

Table 3: Collection dates and locations for 34 Russian wheat aphid isolates collected from noncultivated grasses in northeastern Colorado in 2011 characterized for biotypic diversity.

Colony number[a]	Sampling date	Site
M1	07/07/2011	9 b
M2	07/07/2011	9 j
M3	07/07/2011	9 m
P5	07/14/2011	3 a
P6	07/14/2011	3 b
P9	07/14/2011	3 h
M10	07/21/2011	9 a
M11	07/21/2011	9 d
M12	07/21/2011	11 c
M13	07/21/2011	13 f
P14	07/28/2011	5 b
P16	07/28/2011	6 c
P17	07/28/2011	1 a
P18	07/28/2011	1 b
P19	07/28/2011	1 g
P20	07/28/2011	1 h
P21	07/28/2011	3 b
P22	07/28/2011	3 c
P23	07/28/2011	3 e
P24	07/28/2011	3 g
P25	07/28/2011	3 l
M26	08/04/2011	9 a
M27	08/04/2011	9 c
M28	08/04/2011	9 f
M29	08/04/2011	9 i
M30	08/04/2011	9 r
M31	08/04/2011	9 u
M32	08/04/2011	11
M33	08/04/2011	12 a
M36	08/04/2011	15 a
M37	08/04/2011	15 b
M38	08/04/2011	15 c
M39	08/04/2011	15 d
M40	08/04/2011	15 e

[a] the upper case letter before the colony number indicates from where it was collected, M= Montane site, P= Prairie site.

[b] the letter after the site number identifies each different colony established at the same site on the same date.

Table 4: Plant differentials used to determine Russian wheat aphid biotypes for isolates collected from noncultivated grass hosts in prairie and montane environments in northeastern Colorado in 2010 and 2011

Plant resistance source	Gene designation and/or PI number	Expressed biotype resistance[a]	Reference
Wheat			
CO03797 (*)	*Dn*1	1	Haley et al. (2004)
CO03804	*Dn*2	1	Haley et al. (2004)
CO03811	*dn*3	1	Haley et al. (2004)
Yumar (*)	*Dn*4, PI 605388	1	Quick et al. (2001)
CO950043	*Dn*5	1	Haley et al. (2004)
CO960223 (*)	*Dn*6	1	Haley et al. (2004)
94M370	*Dn*7	1,2	Haley et al. (2004)
Karee-Dn8	*Dn*8, PI 634775	1	Tolmay et al. (2006)
Betta-Dn9	*Dn*9, PI 634770	1	Tolmay et al. (2006)
KS94WGRC29	PI 586954	1	Martin and Harvey (1997)
Stanton	PI 617033	1	Collins et al. (2005)
STARS 9302W	PI 572289	1	Baker et al. (1994)
KS92WGRC25	PI 574490	1	Martin and Harvey (1995)
CI2401 (*)	PI 9781	1,2	Dong et al. (1997)
PI 626197	PI 626197	1,2	USDA, ARS, National Genetic Resources Program. (1)
PI 625139	PI 625139	1,2	USDA, ARS, National Genetic Resources Program. (2)
PI 626580-4	PI 626580-4	1,2	Valdez et al. (2012)
Hatcher	*Dn*4, PI 638512	1	Haley et al. (2005)
Yuma (*)	Susc. check, PI 559720		McVey and Long (1993)
CO08RWA50 (*)	*Dn*7, PI 664301	1,2	USDA, ARS, National Genetic Resources Program. (3)
CO03765 (*)	PI 659322	1	Nkongolo et al. (2011)
Barley			
Otis	Susc. check, CIho 7557		Heisel et al. (1986)
Stoneham (*)	PI 641940	1,2	Mornhinweg et al. (2012a)
Sidney	PI 641939	1,2	Mornhinweg et al. (2009)

(*) indicates a plant differential used in screening both 2010 and 2011 isolates.

[a] 1, resistant to RWA1; 2, resistant to RWA2 (Weiland 2008).

Sixteen flats, each measuring 27.5 x 27.5 cm, were planted with four rows containing six plant differentials per row and five seeds for each differential. Plant differentials were randomized within each flat (Figure 6). After planting, flats were placed in cages covered with fine mesh (0.2 x 0.2 mm), white polyester organza to prevent contamination by non colony aphids and parasitoids (Figure 7). At the single leaf stage, plants were infested by placing 10 aphids from a single colony next to each seedling in the flat. Each flat was infested with a different isolate. Flats were held in a growth room under supplemental artificial light with a 16-hour photoperiod with daytime temperatures approximately 25° (±2 °C) and night temperatures approximately 20° (±2 °C). Once the RWA1 susceptible wheat cultivar Yuma and barley cultivar Otis were killed, all plants were rated for leaf chlorosis and leaf rolling (Figure 8). Leaf chlorosis was rated on a one to nine scale, with one signifying a healthy plant and nine signifying a severely streaked and dead, or dying, plant (Webster et al. 1987). Leaf rolling was rated on a one to three scale, with one signifying no leaf rolling, two, one or more leaves conduplicately folded and three signifying one or more leaves convolutedly rolled (Burd et al. 1993). Leaf chlorosis scores of one to three and/or a leaf rolling score of one were considered resistant, leaf chlorosis scores of four to six and/or a leaf rolling score of two were considered to have intermediate resistance, and leaf chlorosis scores of seven to nine and/or a leaf rolling score of three were considered susceptible (Weiland 2006). These are the categories used in the virulence profile table. Six replicates of the 14 isolates from 2010 were biotyped from December 2010 to September 2011.

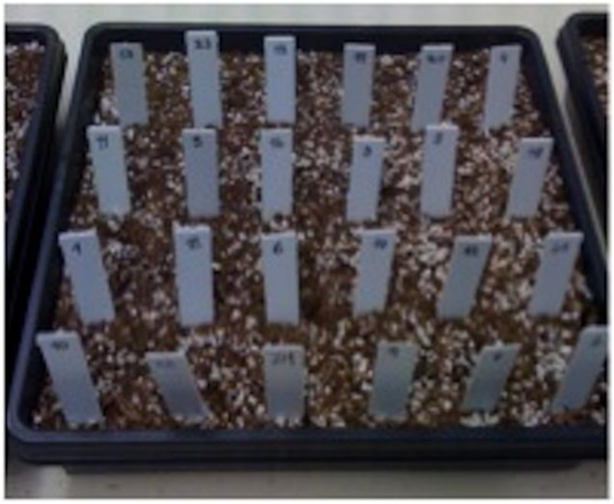

Figure 6: Arrangement of wheat and barley differentials used to biotype Russian wheat aphid colonies collected from noncultivated hosts in northeastern Colorado in 2010

Figure 7: Organza cages used to prevent cross contamination among flats in figure 6

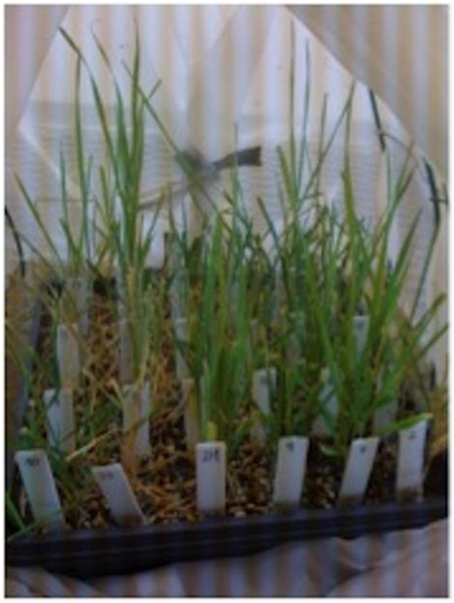

Figure 8: Plant differentials to be rated for chlorosis (1-9 scale) and leaf rolling (1-3 scale) in order to biotype Russian wheat aphid colonies from noncultivated hosts in northeastern Colorado in 2010. Ratings were taken when susceptible checks had a chlorosis rating of 9.

In order to determine the biotypic status of the 34 isolates from 2011, the number of plant differentials used for screening the isolates had to be reduced to eight, due to the lack of space in the growth room to test all isolates. These lines were selected from those used to biotype the isolates from 2010 (Table 4). Correlations values between the 24 plant differentials, mean values of leaf chlorosis and standard deviations of each plant differential, all obtained with the statistical program R, were used to select the eight plant differentials for biotyping the 34 isolates from 2011. Known biotypes RWA1 and RWA2 were included as control.

The eight plant differentials used for biotyping 2011-isolates were selected using Figure 11, at the clustering level on the Y-axis where the plant differentials are grouped in 8 different groups of similar resistance. At each clustering level, I first compared the correlation values between each plant differential (see Appendix B, 2010 isolates: plant differentials). For clusters with more than two plant differentials per cluster, I selected the plant differentials that were highly correlated. I continued with the selection process from those selected plant differentials. The statistic values used for narrowing the selection of plant differentials were the following: mean values of leaf chlorosis and standard deviation of each plant differential at each clustering level (see Appendix B, 2010 isolates: plant differentials). In this second step, I compared the mean values of leaf chlorosis and standard deviation of the plant differential selected in the previous step. Between plant differentials with similar mean values of leaf chlorosis, I selected the ones with smaller standard deviations. The source of resistance was the third selection criteria that I used for selecting plant differentials with similar mean values of leaf chlorosis and similar standard deviation within a given clustering level. I also used the third selection criterion for clustering levels where the mean values of leaf chlorosis of the plant differentials were significantly different. In this third step, I compared the source of resistance of the plant differentials and selected those with most commonly used resistance genes used in plant breeding programs. Finally, I also considered the species variability of the plant differentials, and included one line of barley in the selected eight plant differentials.

In summary, the objective was to select eight plant differentials that were genetically diverse, had different resistant genes, and that represented the range of chlorosis scores observed in the 2010 experiment. The eight plant differentials selected from these eight groups were: CO960223, CO08RWA50, CI2401, CO03797, CO03765, Stoneham, Yuma, and Yumar (Table 4).

Thirty-six trays with 32 square cells, 4x8 configuration, measuring 13.75 cm x 27.5 cm, were planted with four rows containing eight plant differentials per row and five seeds for each one. Differentials were randomized within each flat (Figure 9). After planting, flats were placed in cages covered with individual fine mesh (0.2 x 0.2 mm), white polyester organza material cages to prevent aphid contamination (Figure 10). The methods for infestations, test environment and damage rating were the same as used for the isolates from 2010. Six replicates of 34 isolates from 2011 were biotyped from January 2012 to February 2013.

I did not do a virulence profile nor analyze the leaf rolling data for the 2011 isolates because I just wanted to see if there was variability within the isolates by establishing the three different groups of damage with the leaf chlorosis data.

V. Data analysis

Data from all Russian wheat aphid colony screenings were analyzed using SAS version 9.01 (SAS Institute 2010). The PROC MIXED routine was used to test differences between aphid isolates in leaf chlorosis and leaf rolling scores by treating plant differentials as fixed effects and aphid isolates as random effects. Degrees of freedom were determined using the Kenward-Roger method. The Slice statement was used to test if there was a significant difference (P-value < 0.05, $\alpha = 0.05$) between isolates in leaf chlorosis scores for each plant differential.

The performance of Russian wheat aphid isolates within plant differentials can be compared by the amount of damage that each isolate produced on each plant differential. (Puterka 1992). The amount of damage, in this case leaf chlorosis scores, can be grouped into different levels of damage, an create a profile of the damage, or virulence. A virulence profile was constructed for each of the 14 isolates from 2010, and then the isolates were compared in between each other and with RWA1 and RWA2 profiles. The gplots procedure from the statistical program R was used to cluster the isolates based on similarities of responses, and graphically depict the results. Mean chlorosis and leaf rolling ratings were obtained for the 14 isolates from 2010. Clustering of the isolates based on similarities of responses on leaf chlorosis and leaf rolling damage was obtained for the 14 isolates from 2010. Clustering of the isolates based on similarities of responses on leaf chlorosis was obtained for the 34 isolates from 2011.

Figure 9: Arrangement of wheat lines and barley differentials used to biotype Russian wheat aphid colonies collected from noncultivated hosts in northeastern Colorado in 2011

Figure 10: Organza cages used for the flats of figure 9

RESULTS

Differences in virulence patterns were observed among the 14 isolates from 2010 for leaf chlorosis (Table 5 and Figure 11) and leaf rolling (Figure 12). Susceptible entries displayed expected ratings across all isolates with leaf chlorosis scores ranging from 7.4 to 8.9 (Table 5) and leaf rolling scores ranging from 2.7 to 3.0 (Table 6). Plant differential by biotype interactions occurred for both leaf chlorosis and leaf rolling ($F_{345, 1840}= 2.48$, $P < 0.0001$ and $F_{345, 1840}= 1.75$, $P < 0.0001$, respectively).

The mean values of leaf chlorosis (Table 5) were used to generate a heatmap (Figure 11, see Appendix B: 2010 isolates: leaf chlorosis), and also to create a virulence profile for each isolate (Table 8). Resistance to leaf rolling is emphasized in wheat in plant breeding programs (Scott Haley, Department of Soil and Crop Sciences, Colorado State University, personal communication). However, leaf chlorosis is the best measurement that can be used to create the virulence profiles because it differentiates more biotypes by providing more information about the damaged produced by each biotype than either plant stunting or leaf rolling (Puterka et al. 1992).

The isolates are grouped in three main clusters of similar leaf chlorosis damage (Figure 11). The group containing isolates M5, P14, P6, and P7 produced damage similar to RWA2, where isolate M5 was from a montane site, and the rest of the isolates from prairie sites. The second group contained isolates M2, M8, and M9 that produced damage similar to RWA1, and were all from montane sites. The third group contained isolates M3, M4, M10, M11, M12, M13, and P15 that produced an intermediate level of damage, where isolate P15 was from a prairie site, and the rest of the isolates from montane sites.

The mean values of leaf rolling (Table 6) were used to generate a heatmap (Figure 12). The isolates are grouped in three main clusters of similar leaf rolling damage (Figure 12). The group containing the isolates P14, P6, and P7 produced damage similar to RWA2. The second group contained the isolates M8 and M9 that produced damage similar to RWA1. The third group contained isolates M5, M12, M13, M3, M2, P15, M11, M4 and M10 that produced an intermediate level of damage.

In 2010, a total of 23 Russian wheat aphid isolates were established, 19 isolates were from montane sites, and four isolates were from prairie sites. Three isolates from montane sites with damage similar to RWA1 were determined using the 24 wheat and barley lines. The initial differential screening determined nine RWA1 isolates from montane sites, making a total of 12 isolates from montane sites with damage similar to RWA1 (Table 7).

Table 5: Mean leaf chlorosis scores (1 = healthy plant and 9 = dead plant) of 24 plant differentials infested with Russian wheat aphid isolates established in 2010 from collections from noncultivated grasses in northeastern Colorado montane and prairie environments

Plant resistance source	Isolates															
	RWA1	M2	M3	M4	M5	P6	P7	M8	M9	M10	M11	M12	M13	P14	P15	RWA2
CO03797 (Dn1)	6.6	4.6	5.9	6.5	7.9	6.0	4.9	5.7	5.5	5.9	6.5	6.8	6.8	7.2	6.4	6.1
CO03804 (Dn2)	6.5	6.1	7.3	7.4	7.3	7.6	7.9	5.6	5.6	6.7	8.2	7.0	6.6	8.1	6.9	7.1
CO03811 (dn3)	6.5	7.1	7.4	7.7	8.5	7.4	7.9	6.9	6.8	7.7	8.0	7.6	7.5	8.7	6.9	8.0
Yumar (PI 605388)	5.7	6.5	6.3	6.7	7.0	7.2	7.8	5.6	5.1	7.5	7.1	5.9	6.4	8.7	6.4	8.0
CO950043 (Dn5)	5.2	5.6	7.1	6.5	7.6	7.5	7.9	5.9	5.5	6.4	6.9	6.9	6.8	8.8	6.9	8.3
CO960223 (Dn6)	4.5	3.7	3.6	3.8	5.9	6.7	7.1	5.9	3.6	4.3	4.0	4.9	5.1	8.6	5.0	7.7
94M370 (Dn7)	4.4	3.1	3.0	3.9	5.2	3.1	3.1	4.3	3.9	3.5	3.7	3.7	4.6	4.7	3.5	3.2
Karee-Dn8 (PI 634775)	7.2	4.6	5.8	7.5	7.9	5.6	5.3	6.5	6.2	6.5	5.2	6.5	7.5	7.2	6.6	4.6
Betta-Dn9 (PI 634770)	6.2	5.1	5.7	6.0	7.2	6.0	6.1	7.1	6.6	5.6	5.6	6.2	6.9	5.9	5.6	5.0
KS94WGRC29 (PI 586954)	4.6	3.2	3.3	4.0	4.9	8.0	8.2	4.3	4.0	3.6	3.9	3.3	5.2	8.3	4.4	8.0
Stanton (PI 617033)	6.2	7.2	7.4	7.7	8.2	8.6	8.2	7.1	5.3	7.7	7.8	7.3	7.3	8.8	7.1	8.4
STARS 9302W (PI 572289)	6.2	4.3	4.1	4.9	5.5	6.1	5.5	5.8	5.7	4.5	4.7	5.2	5.6	6.5	4.3	5.1
KS92WGRC25 (PI 574490)	7.4	6.0	6.3	6.6	7.6	6.8	6.6	6.8	6.2	6.2	5.9	7.4	6.7	7.0	6.8	6.4
CI2401 (PI 9781)	4.2	3.9	5.6	4.1	5.5	4.2	4.2	4.3	4.2	4.7	5.5	4.8	4.6	5.7	3.9	4.0
PI626197	4.7	4.6	5.0	5.7	5.9	4.4	4.3	5.8	5.0	5.1	5.6	5.1	5.5	5.4	5.1	5.2
PI625139	5.9	5.9	5.2	6.0	6.4	6.3	5.6	7.8	6.0	5.1	6.2	5.8	6.0	6.3	5.4	5.2
PI626580-4	4.1	3.8	4.0	4.0	5.5	3.1	3.9	4.0	4.0	4.0	4.3	4.7	4.7	4.2	3.3	5.4
Hatcher (PI 638512)	6.3	6.2	6.2	6.9	7.6	7.5	7.9	5.7	5.3	7.3	7.2	6.4	6.7	8.8	6.6	7.7
Yuma (PI 559720)	8.0	7.6	8.1	8.4	8.5	8.2	7.4	8.0	7.6	8.5	8.3	8.4	8.6	8.9	7.8	7.9
CO08RWA50 (PI 664301)	3.6	2.8	3.5	3.4	4.1	2.7	3.5	4.3	3.9	3.7	3.5	3.4	3.4	3.5	3.2	3.3
CO03765 (PI 659322)	5.5	4.2	6.1	5.9	5.7	5.2	5.3	4.4	5.7	6.1	6.4	6.0	5.1	6.0	6.2	6.7
Otis (CIho 7557)	8.5	8.6	7.7	8.2	8.6	7.9	8.2	8.2	8.4	8.1	8.5	8.4	8.3	8.3	8.2	7.5
Stoneham (PI 641940)	5.3	4.9	5.2	5.2	6.8	5.3	4.9	6.4	5.5	4.4	5.1	5.2	5.7	5.3	5.3	5.4
Sidney (PI 641939)	5.5	4.1	3.8	4.9	5.2	3.0	4.5	5.7	4.1	4.5	4.2	3.9	4.7	4.5	4.7	3.4

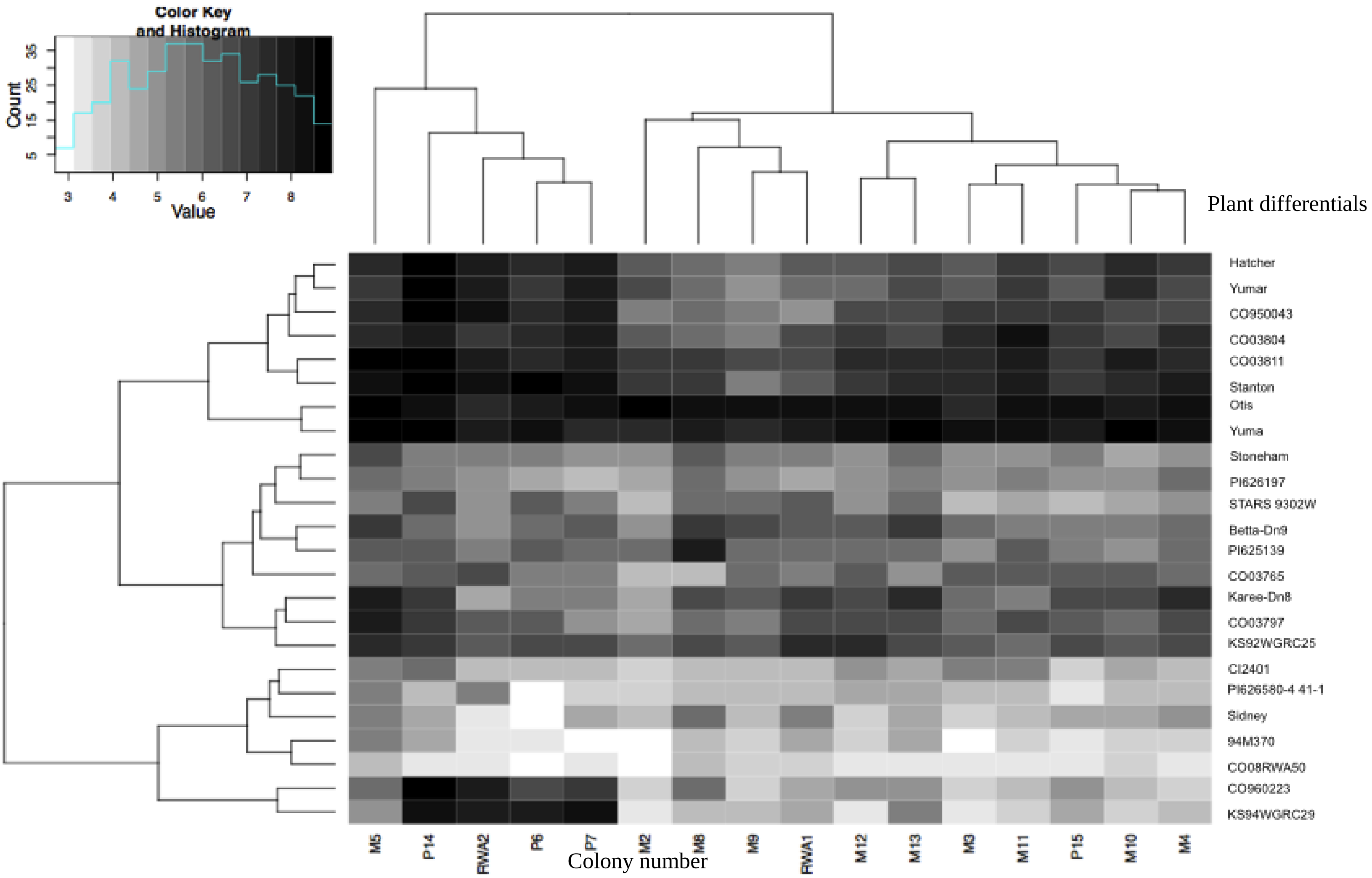

Figure 11: Mean chlorosis ratings[a] and clustering of the isolates based on similarities of responses for 2010 Russian wheat aphid isolates collected from noncultivated grasses in northeastern Colorado prairie and montane environments after artificial infestation

[a] Plant chlorosis rating based on a white (no damage) to black (100% chlorosis) scale.

Table 6: Mean leaf rolling scores (1 = flat and 3 = tightly rolled) of 24 plant differentials infested with Russian wheat aphid isolates established in 2010 from collections from noncultivated grasses in northeastern Colorado montane and prairie environments

Plant resistance source	Isolates															
	RWA1	M2	M3	M4	M5	P6	P7	M8	M9	M10	M11	M12	M13	P14	P15	RWA2
CO03797 (Dn1)	2.3	2.1	2.0	2.1	2.6	1.8	1.9	2.3	2.3	2.2	2.2	2.3	2.3	2.4	2.3	2.2
CO03804 (Dn2)	2.8	2.8	2.9	3.0	2.9	3.0	3.0	2.4	2.5	2.8	2.9	2.9	2.9	2.9	3.0	2.9
CO03811 (dn3)	2.6	2.9	2.8	3.0	3.0	2.9	3.0	2.9	2.8	3.0	3.0	3.0	3.0	3.0	2.9	3.0
Yumar (PI 605388)	2.4	2.8	3.7	3.0	3.0	2.9	3.0	2.5	2.3	3.0	2.8	2.8	2.8	3.0	2.9	3.0
CO950043 (Dn5)	2.0	2.6	2.6	2.7	2.7	2.8	2.9	2.3	2.0	2.5	2.9	2.8	2.8	3.0	2.5	3.0
CO960223 (Dn6)	1.9	2.1	2.2	2.0	2.4	2.8	2.9	2.5	1.8	2.1	1.8	2.3	2.4	3.0	2.2	23.0
94M370 (Dn7)	1.4	1.6	1.4	1.6	2.2	1.4	1.3	1.6	1.6	1.5	1.5	1.6	1.7	1.7	1.5	1.3
Karee-Dn8 (PI 634775)	2.3	1.7	2.3	2.3	2.7	2.0	1.8	2.4	2.3	2.4	2.0	2.5	2.5	2.5	2.3	1.9
Betta-Dn9 (PI 634770)	1.8	1.9	1.8	2.0	2.3	1.8	1.8	2.3	2.2	1.9	1.9	2.0	2.3	2.1	1.8	1.9
KS94WGRC29 (PI 586954)	2.3	1.4	1.5	2.1	1.9	2.9	3.0	1.9	1.6	1.8	1.8	1.5	2.1	2.9	2.1	3.0
Stanton (PI 617033)	2.5	2.8	3.0	3.0	3.0	3.0	2.9	2.6	2.2	3.0	3.0	3.0	3.0	3.0	3.0	3.0
STARS 9302W (PI 572289)	2.3	2.0	1.7	1.7	2.1	2.6	2.1	2.1	2.1	1.8	1.7	2.0	2.1	2.4	1.7	2.1
KS92WGRC25 (PI 574490)	2.6	2.2	2.3	2.1	2.5	2.2	2.2	2.3	2.1	2.1	1.7	2.5	2.2	2.4	2.3	2.2
CI2401 (PI 9781)	2.0	1.9	2.1	1.8	2.1	1.8	1.9	2.0	2.0	2.0	1.8	2.0	1.9	2.1	2.1	1.9
PI626197	2.0	2.1	1.9	2.0	2.3	1.7	1.9	2.0	1.9	2.0	2.0	1.9	2.1	2.2	2.2	2.0
PI625139	2.0	2.4	2.1	1.9	2.6	2.3	2.3	2.6	2.2	2.1	2.2	2.4	2.4	2.6	2.5	2.2
PI626580-4	1.7	1.5	1.7	1.4	2.0	1.7	1.8	1.7	1.6	1.7	1.8	1.9	2.0	1.7	1.5	1.8
Hatcher (PI 638512)	2.4	2.8	2.8	3.0	2.9	2.8	3.0	2.3	2.2	2.8	3.0	2.8	2.9	3.0	2.9	3.0
Yuma (PI 559720)	3.0	2.8	3.0	3.0	3.0	3.0	2.9	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
CO08RWA50 (PI 664301)	1.6	1.6	1.8	1.8	1.7	1.0	1.5	1.8	1.8	1.9	1.8	1.7	1.8	1.7	1.3	1.4
CO03765 (PI 659322)	2.4	2.0	2.6	2.4	2.5	2.2	2.3	2.1	2.3	2.8	2.6	2.5	2.4	2.5	2.6	2.8
Otis (CIho 7557)	3.0	3.0	3.0	2.7	3.0	3.0	2.9	2.8	3.0	3.0	2.9	3.0	3.0	2.9	3.0	2.9
Stoneham (PI 641940)	2.0	2.2	1.8	2.1	2.6	2.0	2.1	2.3	2.0	2.0	2.2	2.3	2.2	2.2	2.2	2.2
Sidney (PI 641939)	1.7	2.0	1.7	1.8	1.9	1.5	1.6	2.1	1.8	2.0	1.9	1.8	2.2	1.6	1.8	1.6

[1,2,3]Table 7: Distritubion into leaf chlorosis damage categories of Russian wheat aphid isolates from collections made in 2010 from noncultivated grasses in northeastern Colorado montane and prairie environments

Site	Low damage[a]	Intermediate damage[b]	Heavy damage[c]	Total number of isolates per site
Montane	12 (63.2%)	6 (31.6%)	1 (5.2%)	19 (100%)
Prairie	0	1 (25%)	3 (75%)	4 (100%)
		2010 total number of isolates		23

[1] Burd et al, 1998.
[2] See Table 4 for descriptions of differentials.
[3] See Figure 11 for heat map comparisons of means and Appendix B for correlation matrix.
[a] Low amount of damage, similar to that expected by biotype RWA1 on plants expressing Dn4 resistance.
[b] Intermediate level of damage.
[c] Heavy damage, similar to that expected from biotype RWA2 feeding on susceptible plants or plants expressing Dn4 resistance .

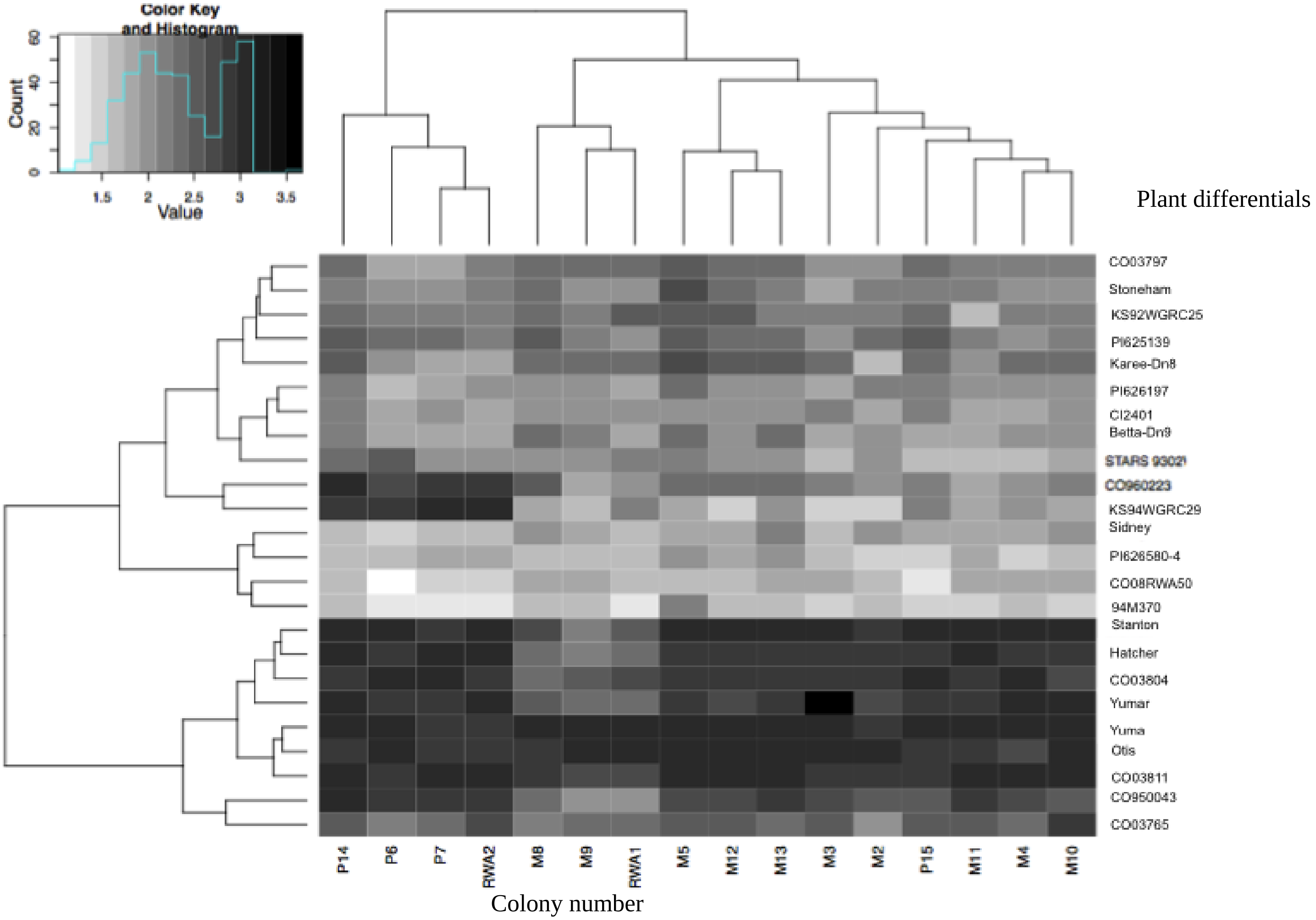

Figure 12: Mean rolling ratings[a] and clustering of the isolates based on similarities of responses for 2010 Russian wheat aphid isolates collected from noncultivated grasses in northeastern Colorado prairie and montane environments after artificial infestation of 24 plant differentials

[a] Leaf rolling rating based on a white (no leaf rolling) to black (one or more leaves convolutedly rolled) scale.

Isolates P6 and P7, collected in 2010, produced similar damage compared to the RWA2 biotype (Table 8). Isolate P6 produced low amount of damage to PI626580-4 (p=0.02), and intermediate damage to CO03765 (p=0.044), whereas RWA2 produced intermediate damage to PI626580-4, and low amount of damage to CO03765.

Isolates M5 and P14, also collected in 2010, produced more damage than the RWA2 biotype (Table 8). Isolate M5 produced heavy damage to CO03797 (p=0.015), Karee-Dn8 (p=6.37E-06), Betta-Dn9 (p=0.002), and intermediate damage to 94M370 (p=0.005) and Sidney (p=0.011), whereas RWA2 biotype produced intermediate damage to CO03797, Karee-Dn8, and Betta-Dn9, and low amount of damage to 94M370 and Sidney. On the other hand, isolate M5 produced intermediate damage to CO960223 (p=0.0002) and KS94WGRC29 (p=2.87E-05), whereas RWA2 biotype produced heavy damage to CO960223 and KS94WGRC29. Isolate P14 produced heavy damage to Karee-Dn8 (p=0.0005) and intermediate damage to 94M370 (p=0.005), whereas RWA2 biotype produced intermediate damage to Karee-Dn8 and low damage to 94M370. Isolates M5 and P14 are going to be retested in order to confirm these results, and to determine if they should be designated as new biotypes.

Table 8: Virulence profile of each 2010 Russian wheat aphid isolate collected from noncultivated grasses in northeastern Colorado prairie and montane environments based on chlorosis rating scores: resistant (R) 1-3, intermediate (I) 4-6, and susceptible (S) 7-9

Plant resistance source	Isolate															
	RWA1	M2	M3	M4	M5	P6	P7	M8	M9	M10	M11	M12	M13	P14	P15	RWA2
CO03797 (Dn1)	S	I	I	I	S	I	I	I	I	I	I	S	S	S	I	I
CO03804 (Dn2)	S	I	S	S	S	S	S	I	I	S	S	S	S	S	S	S
CO03811 (dn3)	I	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Yumar (PI 605388)	I	I	I	S	S	S	S	I	I	S	S	I	I	S	I	S
CO950043 (Dn5)	I	I	S	I	S	S	S	I	I	I	S	S	S	S	S	S
CO960223 (Dn6)	I	I	I	I	I	S	S	I	I	I	I	I	I	S	I	S
94M370 (Dn7)	I	R	R	I	I	R	R	I	I	I	I	I	I	I	R	R
Karee-Dn8 (PI 634775)	S	I	I	S	S	I	I	S	I	S	I	I	S	S	S	I
Betta-Dn9 (PI 634770)	I	I	I	I	S	I	I	S	S	I	I	I	S	I	I	I
KS94WGRC29 (PI 586954)	I	R	R	I	I	S	S	I	I	I	I	R	I	S	I	S
Stanton (PI 617033)	I	S	S	S	S	S	S	S	I	S	S	S	S	S	S	S
STARS 9302W (PI 572289)	I	I	I	I	I	I	I	I	I	I	I	I	I	S	I	I
KS92WGRC25 (PI 574490)	S	I	I	S	S	S	S	S	I	I	I	S	S	S	S	I
CI2401 (PI 9781)	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
PI626197	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
PI625139	I	I	I	I	I	I	I	S	I	I	I	I	I	I	I	I
PI626580-4	I	I	I	I	I	R	I	I	I	I	I	I	I	I	R	I
Hatcher (PI 638512)	I	I	I	S	S	S	S	I	I	S	S	I	S	S	S	S
Yuma (PI 559720)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CO08RWA50 (PI 664301)	I	R	R	R	I	R	R	I	I	I	R	R	R	R	R	R
CO03765 (PI 659322)	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	S
Otis (CIho 7557)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Stoneham (PI 641940)	I	I	I	I	S	I	I	I	I	I	I	I	I	I	I	I
Sidney (PI 641939)	I	I	I	I	I	R	I	I	I	I	I	I	I	I	I	R

For the 34 isolates from 2011, I only considered the values of leaf chlorosis for evaluating the isolates, as they explained better the level of damage produced by each isolate (Puterka et al. 1992). Plant differential by biotype interactions occurred for leaf chlorosis ($F_{245,\ 1260}= 3.82$, $P < 0.0001$). The mean values of leaf chlorosis (Table 9) were used to generate the heatmap (Figure 13).

As in 2010, the 34 isolates from 2011 could be placed in three groups, based on damage similarity to RWA1 and RWA2. Isolates producing damage similar to RWA1 included M40, M39, M38, and M32. Isolates with damage intermediate to RWA1 and RWA2 included P19, M30, M12, M26, M36, P17, M13, P20, P18, M27, M31, M10, and M37. Isolates producing chlorosis ratings similar to RWA2 included M11, M29, P23, P24, P25, P6, P22, P21, M33, M28, M1, M2, P16, P5, M3, P9, and P14 (Figure 13).

Collection date and site did not influence damage for either 2010 or 2011 isolates. For example, isolates M5, M2, M3 and M4 from 2010 were all collected from the same montane site, the same date, but they were placed in different virulence groups. From 2011 isolates, isolates M40, M36 and M37 were also collected from the same montane site, the same date, but were placed in the virulent and moderately virulent groups. Unlike the 2010 isolates, none were identified as potential new biotypes.

In 2011, a total of 100 Russian wheat aphid isolates were established, 71 isolates from montane sites, and 29 isolates from prairie sites. Due to the lack of time and space in the growth room, only 34 isolates shown to be not biotype RWA1 in the initial screening were biotyped. From these 34 isolates, 14 were from prairie sites, and 20 were from montane sites. The initial differential screening of the 100 Russian wheat aphid isolates determined one RWA1 isolate from a prairie site and 12 RWA1 isolates from montane sites, making a total of 16 isolates from montane sites and one isolate from a prairie site with damage similar to RWA1 (Table 10).

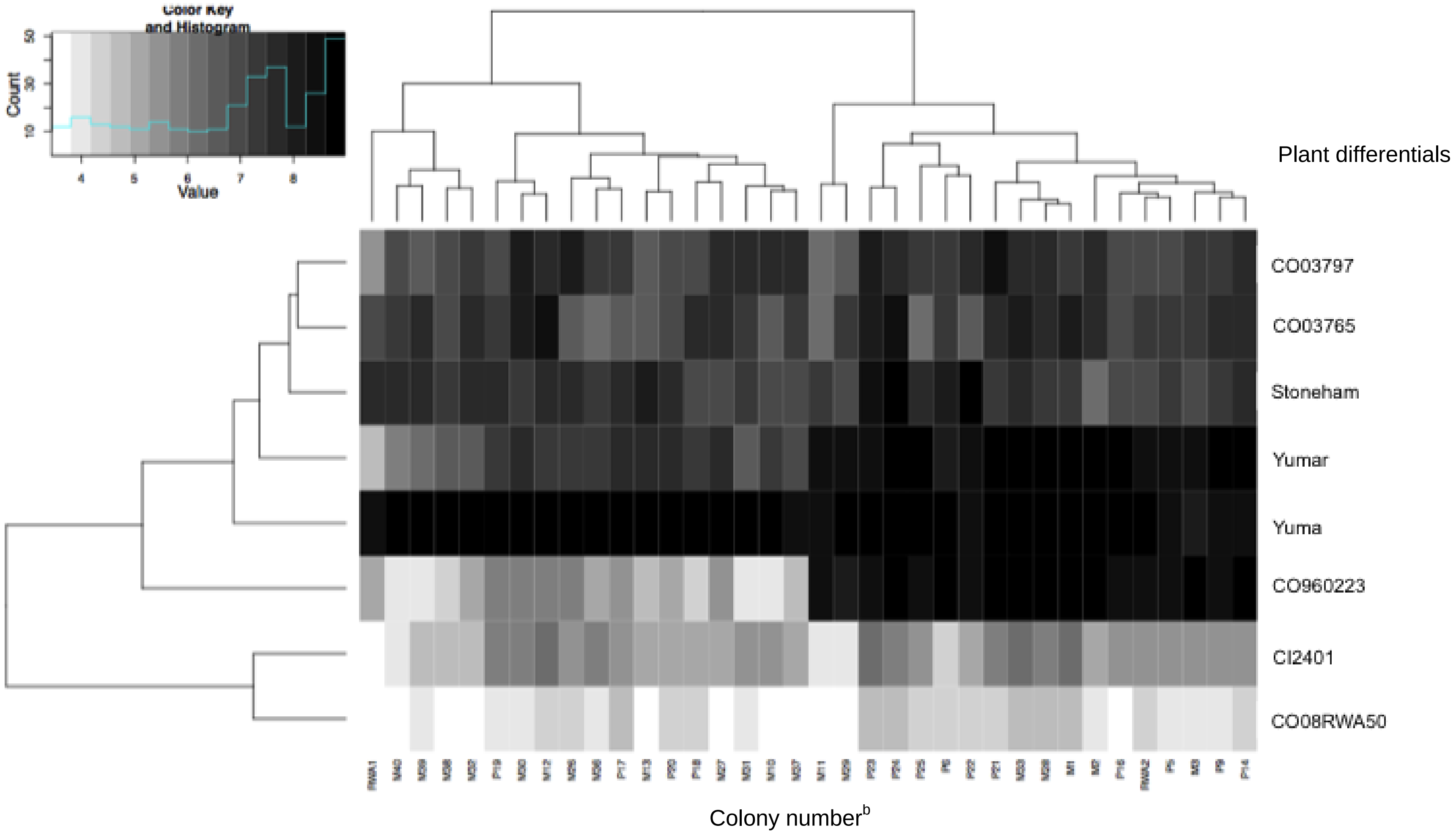

Figure 13: Mean chlorosis ratings[a] and clustering of the isolates based on similarities of responses for 2011 Russian wheat aphid isolates collected from noncultivated grasses in northeastern Colorado prairie and montane environments after artificial infestation of 24 plant differentials.

[a] Plant chlorosis rating based on a white (no damage) to black (100% chlorosis) scale.

Table 9: Mean leaf chlorosis scores (1 = healthy plant and 9 = dead plant) of eight plant differentials infested with Russian wheat aphid isolates established in 2011 from collections from noncultivated grasses in northeastern Colorado montane and prairie environments

Plant resistance source	Isolates																		
	M 1	M 2	M 3	P 5	P 6	P 9	M 10	M 11	M 12	M 13	P 14	P 16	P 17	P 18	P 19	P 20	P 21	P 22	P 23
CO03797	7.48	7.71	7.39	6.87	7.48	7.35	7.58	6.19	7.70	6.60	7.64	7.10	7.44	6.92	7.06	6.98	8.57	7.58	7.95
Yumar	8.66	8.62	8.38	8.29	8.14	8.63	7.17	8.47	7.14	7.77	8.67	8.82	7.78	7.23	7.46	7.56	8.80	8.44	8.28
CO960223	8.87	8.70	8.80	8.53	8.76	8.57	4.07	8.50	5.97	4.77	8.77	8.51	5.33	4.50	5.68	4.96	8.93	8.37	8.57
CI2401	6.28	5.27	5.48	5.44	4.47	5.63	5.40	4.07	6.02	4.97	5.54	5.40	5.58	5.27	5.74	5.08	5.86	5.01	6.31
Yuma	8.90	8.73	8.18	8.55	8.75	8.49	8.78	8.49	8.77	8.73	8.50	8.87	8.90	8.64	8.90	8.70	8.80	8.59	8.81
CO08RWA50	4.73	3.97	4.08	4.17	4.44	3.82	3.74	3.43	4.40	3.52	4.43	3.77	4.56	4.20	4.00	4.23	4.50	4.30	4.73
CO03765	7.88	7.64	7.18	7.33	7.30	7.69	6.65	6.20	8.34	6.61	7.60	7.12	6.59	7.63	7.44	7.00	7.52	6.61	8.02
Stoneham	7.41	6.37	7.00	7.43	7.90	7.32	7.04	7.20	7.54	7.95	7.58	6.98	7.62	7.04	7.68	7.75	7.24	8.97	8.31

Plant resistance source	Isolates																
	P 24	P 25	M 26	M 27	M 28	M 29	M 30	M 31	M 32	M 33	M 36	M 37	M 38	M 39	M 40	RWA1	RWA2
CO03797	7.73	7.40	8.18	7.64	7.78	6.67	8.02	7.53	7.43	7.85	7.28	7.73	7.03	6.41	6.83	5.41	7.00
Yumar	8.77	8.96	7.23	7.57	8.62	8.41	7.59	6.39	6.64	8.79	7.26	6.80	6.74	6.10	5.85	4.91	8.52
CO960223	8.60	8.45	5.73	5.32	8.92	8.22	5.77	4.12	5.04	8.63	5.04	4.68	4.23	4.01	3.90	4.93	8.26
CI2401	5.65	5.61	5.47	5.24	5.86	3.87	5.70	5.42	4.69	6.12	5.87	5.14	4.64	4.77	4.11	3.68	5.35
Yuma	8.72	8.86	8.60	8.63	8.78	8.81	8.80	8.76	8.81	8.73	8.89	8.47	8.85	8.63	8.81	8.43	8.70
CO08RWA50	4.83	4.23	4.30	3.70	4.80	3.72	4.13	3.97	3.58	4.85	3.90	3.80	3.70	4.13	3.48	3.70	4.37
CO03765	8.29	6.28	6.60	7.85	7.74	7.13	7.93	7.36	7.50	7.92	6.24	7.17	7.08	7.65	7.22	7.05	7.24
Stoneham	8.95	7.63	7.79	6.93	7.27	6.96	7.25	7.43	7.60	7.79	7.37	6.94	7.24	7.60	7.58	7.63	6.82

[1,2,3]Table 10: Distribution into damage categories of Russian wheat aphid isolates from collections made in 2011 from noncultivated grasses in northeastern Colorado montane and prairie environments

Site	Low damage[a]	Intermediate damage[b]	Heavy damage[c]	Total number of isolates per site
Montane	16 (50%)	9 (28.1%)	7 (21.9%)	32[d] (100%)
Prairie	1 (6.7%)	4 (26.7%)	10 (66.6%)	15[e] (100%)
		2011 total number of isolates biotyped		47

[1] Burd et al, 1998.
[2] See Table 4 for descriptions of differentials.
[3] See Figure 13 for heat map comparisons of means and Appendix B: for correlation matrix.
[a] Low amount of damage, similar to that expected by biotype RWA1 on plants expressing Dn4 resistance.
[b] Intermediate level of damage.
[c] Heavy damage, similar to that expected from biotype RWA2 feeding on susceptible plants or plants expressing Dn4 resistance .
[d] 32 montane isolates= 20 isolates biotyped with full set of differentials+12 isolates determined with initial differential screening.
[e] 15 prairie isolates= 14 isolates biotyped with full set of differentials+1 isolate determined with initial differential screening.

DISCUSSION

The results of this study showed the importance of alternate grass hosts for the development of biotypes. At all sites from montane environments, the isolates were grouped into the three main damage level clusters, indicating the high variability in the populations of Russian wheat aphid presents on those sites. As mentioned by Shufran et al. (2011), is very likely that the source of these new biotypes is genetic recombination during the holocycle, though to confirm the occurrence of holocycly in Russian wheat aphid populations it would be necessary to find male Russian wheat aphids and/or viable overwintering eggs locally. I conducted limited searches for eggs and male Russian wheat aphids at montane sites following the method of Pucherelli (2010), but without success.

It is unclear if Russian wheat aphids present at high elevations are local or immigrants from lower elevations. Pucherelli (2010) suggested that Russian wheat aphid populations in the Cache La Poudre River canyon are maintained by both immigrant aphids and from local metapopulations within the canyon. Due to lower temperatures and variable host quality, aphids may encounter selective pressures different from those experienced in wheat fields, and may require holocycly for year round survival. This is consistent with the discovery of Russian wheat aphid fundatrices resulted from cyclical parthenogenesis at Dove Creek, Colorado (elevation 2,058 m) by Puterka et al. (2012). Cyclical parthenogenesis in Russian wheat aphid population was associated with wild grasses, which maintain Russian wheat aphid populations throughout the summer and offer a stable habitat for the sexual phase of cyclical parthenogenesis. Puterka et al. (2012) concluded that the wheat agroecosystem is not compatible with cyclical parthenogenesis because the gap between wheat harvest in June and July and next wheat planting in September and October occurs at the critical time when sexual morphs occur in August.

Comparing the results from Table 7 and Table 10, it is possible to infer that the montane collections were more biotypically diverse than the prairie collections, for both 2010 and 2011 sampling years. The isolates established from montane sites were distributed in all three damage groups, while the isolates from prairie sites were placed in the heavy damage and intermediate damage groups, with only one isolate from 2011 in the low damage group. However, these results could be affected by the fact that there were more montane isolates than prairie isolates. In order to obtain a better estimation of the biotypic diversity of each environment, a montane subset should be selected at random to make this comparison.

Overall, there were more isolates from montane sites in the low damage group, and more isolates from prairie sites in the heavy damage group, for both years. These results would confirm the assumptions made by Pucherelli (2010) and Puterka et al. (2012) regarding holocycly being more likely in high elevations environments and a source of biotypic diversity through sexual recombination. Even though a high presence of Russian wheat aphid populations producing heavy damage would be expected in montane environments, due to the possibility of sexual reproduction and the pressure excerted by the harsh habitat conditions, it might be possible that RWA1 populations are better adapted to survive at high elevations, explaining the higher presence of isolates producing low amount of damage. Isolates producing heavy damage were more abundant in prairie environments. The presence of alternate grass hosts during the summer, and milder winter conditions than the ones occurring at higher elevations might play an important role in Russian wheat aphid populations in prairie environments, favoring the abundance of RWA2 populations.

The isolates from 2010, clustered in the three main groups of damage for leaf chlorosis, were almost the same as the ones clustered in the three main groups of damage for leaf rolling, for the same year. The group containing the isolates M5, P14, P6, and P7 that produced leaf chlorosis damage similar to RWA2 was similar to the group that produced leaf rolling damage similar to RWA2, with the exception of isolate M5 that was in the intermediate damage group for leaf rolling. The group containing the isolates M2, M8, and M9 that produced leaf chlorosis damage similar to RWA1 was similar to the group that produced leaf rolling damage similar to RWA1, with the exception of isolate M2 that was in the intermediate damage group for leaf rolling. These results showed that both measurements, leaf rolling and leaf chlorosis, could be used interchangeably to cluster isolates in different groups of damage. Nevertheless, leaf chlorosis would still be needed to differentiate biotypes, because it provides more information about the damaged produced by each isolate.

This study also showed that monitoring Russian wheat aphid for new biotypes is a useful technique, given this research discovered two previously unknown biotypes, isolate M5, collected from a montane site, and isolate P14, collected from a prairie site. Both isolates produce more damage than RWA2 biotype. The presence of an isolate producing heavy damage at high elevations could be explained by mutation, and also by sexual recombination due to holocycly. Pucherelli (2010) attempted to determine alate Russian wheat aphid movement from areas of wheat production to high elevations during the summer, using suction traps located at several sites in the Cache La Poudre River canyon. However, valley breezes coming from high elevations would also deposit alate aphids at lower elevations, explaining the presence of an isolate that produced more damage than RWA2 biotype in prairie environments. Although holocycly in Russian wheat aphid is thought to occur mainly at high elevations, sexual recombination could also occur in prairie environments, and this could also be another reason for the presence of an isolate that produced more damage than RWA2 biotype in prairie environments.

The identification of biotypes and sources of resistance to these biotypes is important in wheat breeding programs (Randolph et al. 2009). The plant differential CO08RWA50, that contains the resistance gene *Dn*7, was highly resistant to all biotypes and thus good candidate resistance source.

CONCLUSIONS

Alternate grass hosts in montane and prairie environments are an important source of variability and means of survival for Russian wheat aphid populations. They are the only source of shelter and food for RWA populations in montane environments, and the means of survival between wheat harvest and fall planting of the next year's crop for the populations present in prairie environments.

The M5 and P14 isolates exhibited virulence profiles unique from known biotypes of Russian wheat aphid in Colorado. These findings support the theory that noncultivated hosts and higher elevations play a role in aphid biotype development. Sampling of alternate hosts in prairie and montane environments should continue in order to monitor the development of new biotypes that might be addressed through modified management practices or breeding strategies.

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APPENDIX I. SAS input.

SAS input 2010 isolates
Screening of Biotype **1**, Biotype **2** over **14** colonies of unknown Biotypes
Bt: **1**= Biotype **1**, **2**= unknown Biotype colony #**M2**, **3**= unknown Biotype colony #**M3**, **4**= unknown Biotype colony #**M4**, **5**= unknown Biotype colony #**M5**, **6**= unknown Biotype colony #**P6**, **7**= unknown Biotype colony #**P7**, **8**= unknown Biotype colony #**M8**, **9**= unknown Biotype colony #**M9**, **10**= unknown Biotype colony #**M10**, **11**= unknown Biotype colony #**M11**, **12**= unknown Biotype colony #**M12**, **13**= unknown Biotype colony #**M13**, **14**= unknown Biotype colony #**P14**, **15**= unknown Biotype colony #**P15**, **16**= Biotype **2**

Rep: repetitions **1**-**6**
Line: plant entries **1**-**24**
Ls: plant chlorosis rating
Lr: leaf rolling rating

```
data rwa;
input bt rep line ls lr;
datalines;
1      1      1      7      2.33
1      1      2      7      2.67
1      1      3      6.2    2.6
1      1      4      5      2
1      1      5      3.75   2
1      1      6      4      2
1      1      7      4.8    1.6
1      1      8      6.6    2.2
1      1      9      7.33   2
1      1      10     4      2
1      1      11     3.5    2
1      1      12     7.67   2.33
1      1      13     5      2
1      1      14     5      2.33
1      1      15     6      2
1      1      16     5.6    2
1      1      17     5      2.33
1      1      18     5      2
1      1      19     7.25   3
1      1      20     3.4    2
1      1      21     6.4    2.6
1      1      22     7.2    3
1      1      23     5      2
1      1      24     5      1.4
1      2      1      6      2.2
```

1	2	2	6.5	3
1	2	3	7.5	3
1	2	4	5	2.4
1	2	5	6.33	2.33
1	2	6	4	2
1	2	7	4.33	1
1	2	8	7.2	2
1	2	9	4	0.8
1	2	10	5	3
1	2	11	4.6	2
1	2	12	5	3
1	2	13	8.25	2.5
1	2	14	4.5	1.5
1	2	15	3	1
1	2	16	5.5	2
1	2	17	3.8	1
1	2	18	5	2
1	2	19	7	3
1	2	20	3.6	1.4
1	2	21	5.4	2.2
1	2	22	8.6	3
1	2	23	6	1
1	2	24	5	1
1	3	1	5.5	2
1	3	2	9	3
1	3	3	8	3
1	3	4	5.6	2
1	3	5	3.2	1.2
1	3	6	2.8	1
1	3	7	4.5	1
1	3	8	8.33	2.33
1	3	9	6.25	2
1	3	10	3.75	1.25
1	3	11	8.25	2.75
1	3	12	7	2.25
1	3	13	9	3
1	3	14	4.5	1.25
1	3	15	6	2.33
1	3	16	4.75	1
1	3	17	5	1.8
1	3	18	6.8	2.4
1	3	19	8.4	3
1	3	20	3.6	1.6
1	3	21	3.75	1.5
1	3	22	8	3
1	3	23	4.2	1.8

1	3	24	4	1
1	4	1	6.75	2.5
1	4	2	4.5	2.5
1	4	3	7.8	3
1	4	4	5.8	2.4
1	4	5	5.4	2.4
1	4	6	6.4	2.6
1	4	7	5.25	1.5
1	4	8	6.5	2.25
1	4	9	4.8	1
1	4	10	7	3
1	4	11	6.75	3
1	4	12	4.2	1.2
1	4	13	6.6	2.6
1	4	14	4	2
1	4	15	3.25	2
1	4	16	4.75	2
1	4	17	4	2
1	4	18	5.8	2
1	4	19	8	3
1	4	20	3.2	1.2
1	4	21	6.67	2.67
1	4	22	9	3
1	4	23	3.8	1.6
1	4	24	4.2	2
1	5	1	7.67	3
1	5	2	5.4	3
1	5	3	3.8	1.8
1	5	4	5	2.4
1	5	5	5.75	2.25
1	5	6	6.25	2.5
1	5	7	4.2	2
1	5	8	8.6	3
1	5	9	9	3
1	5	10	3	2
1	5	11	9	3
1	5	12	7.8	2.8
1	5	13	8	3
1	5	14	3	2.6
1	5	15	4.2	2
1	5	16	7	2.6
1	5	17	4	2
1	5	18	7.5	3
1	5	19	9	3
1	5	20	3	1.5
1	5	21	5.5	3

1	5	22	9	3
1	5	23	5.8	3
1	5	24	8	3
1	6	1	6.5	2
1	6	2	6.75	2.5
1	6	3	5.6	2.4
1	6	4	8	3
1	6	5	6.6	2
1	6	6	3.4	1.4
1	6	7	3.17	1.17
1	6	8	5.75	2
1	6	9	6	2
1	6	10	4.75	2.25
1	6	11	5	2
1	6	12	5.5	2
1	6	13	7.4	2.2
1	6	14	4.4	2
1	6	15	6	2
1	6	16	7.6	2.6
1	6	17	3	1
1	6	18	7.4	3
1	6	19	8.6	3
1	6	20	4.5	2
1	6	21	5	2.4
1	6	22	9	3
1	6	23	7	2.8
1	6	24	6.67	2
2	1	1	4.5	2.25
2	1	2	1	2
2	1	3	3.6	2.2
2	1	4	4	2
2	1	5	2	1
2	1	6	2.2	1.2
2	1	7	1	1
2	1	8	4	2
2	1	9	3	2
2	1	10	1.4	1
2	1	11	3	2
2	1	12	2	1.33
2	1	13	3	1.5
2	1	14	3	2
2	1	15	4	1.6
2	1	16	8.75	3
2	1	17	3	1
2	1	18	4	2
2	1	19	3	2

2	1	20	1	1
2	1	21	6	2.5
2	1	22	9	3
2	1	23	2.75	1
2	1	24	3	2
2	2	1	5	2
2	2	2	6	3
2	2	3	8.33	3
2	2	4	5.8	2.6
2	2	5	4	2
2	2	6	4	2.4
2	2	7	4	1
2	2	8	5.2	1.8
2	2	9	5.33	0.6
2	2	10	4	2
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2	2	12	6.8	2.2
2	2	13	6.8	2.2
2	2	14	5	1.2
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2	2	18	6.6	3
2	2	19	8.2	3
2	2	20	4	1.6
2	2	21	3	1.4
2	2	22	7.2	3
2	2	23	3.2	1.6
2	2	24	5	1
2	3	1	5	2
2	3	2	7	3
2	3	3	7.75	3
2	3	4	6.4	3
2	3	5	5.2	2.6
2	3	6	3	1.67
2	3	7	3.4	2
2	3	8	4.8	2
2	3	9	4	2
2	3	10	3.5	1
2	3	11	7.4	2.8
2	3	12	3.6	2
2	3	13	8	2.6
2	3	14	4	2
2	3	15	4.25	2
2	3	16	5.2	2
2	3	17	3	1

2	3	18	6.4	3
2	3	19	7.4	3
2	3	20	3.2	2
2	3	21	3.2	1.6
2	3	22	8.8	3
2	3	23	4.2	2
2	3	24	4	2
2	4	1	5.6	2.2
2	4	2	7.4	3
2	4	3	9	3
2	4	4	7	3
2	4	5	6.4	3.75
2	4	6	5.75	2.25
2	4	7	3.6	1.6
2	4	8	5.4	1.8
2	4	9	5	2
2	4	10	4	2
2	4	11	8.8	3
2	4	12	5.5	2
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2	4	14	4.4	2
2	4	15	6.5	3
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2	5	3	7	3
2	5	4	7	3
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2	5	12	4	2.5
2	5	13	7	2.2
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2	5	15	5.2	3

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2	5	19	9	3
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2	5	21	6.75	3
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2	5	24	4.33	2.67
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2	6	3	6.6	3
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2	6	21	2	2
2	6	22	8.4	3
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2	6	24	4	2
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16	3	16	4.2	2.2
16	3	17	7.8	2
16	3	18	7.4	3
16	3	19	8.8	3
16	3	20	3.4	1.4
16	3	21	6.5	2.5
16	3	22	7.4	3
16	3	23	4	2
16	3	24	2.6	1.6
16	4	1	6	2

16	4	2	8	3
16	4	3	9	3
16	4	4	6.4	3
16	4	5	8.6	3
16	4	6	7.5	3
16	4	7	3	1
16	4	8	5.4	2
16	4	9	4.67	2
16	4	10	7.6	3
16	4	11	9	3
16	4	12	4.67	2
16	4	13	5.2	2
16	4	14	3.4	2
16	4	15	5	2
16	4	16	4	2
16	4	17	4	2
16	4	18	7.6	3
16	4	19	8.4	3
16	4	20	2.4	1
16	4	21	6.67	2.83
16	4	22	7.8	3
16	4	23	4.2	2
16	4	24	3	2
16	5	1	4.25	2
16	5	2	7.25	3
16	5	3	7.25	3
16	5	4	9	3
16	5	5	9	3
16	5	6	7	2.75
16	5	7	4	1
16	5	8	3.6	1
16	5	9	4.75	2
16	5	10	8.4	3
16	5	11	9	3
16	5	12	5.2	2
16	5	13	7.2	2.8
16	5	14	4.25	2.25
16	5	15	5.6	2.8
16	5	16	8	3
16	5	17	4	2
16	5	18	8.4	3
16	5	19	7.6	3
16	5	20	3	1
16	5	21	7.8	2.8
16	5	22	8	3
16	5	23	7.4	2.8

```
16      5       24      4       2
16      6       1       7       3
16      6       2       7       3
16      6       3       7.2     3
16      6       4       9       3
16      6       5       9       3
16      6       6       9       3
16      6       7       3       3
16      6       8       4       2
16      6       9       5.8     2.2
16      6       10      9       3
16      6       11      8.2     3
16      6       12      7       3
16      6       13      5.6     2
16      6       14      3.4     2
16      6       15      5.67    2
16      6       16      4       3
16      6       17      3.8     2
16      6       18      8.6     3
16      6       19      7.25    3
16      6       20      4       2
16      6       21      5.6     2.6
16      6       22      9       3
16      6       23      6.4     3
16      6       24      3.4     2
;
run;

proc mixed data=rwa;
class bt rep line;
model ls= bt|line/ddfm=kr residual;
random rep rep*bt;
lsmeans bt*line;
slice bt*line/ sliceby=line pdiff;
ods output slicediffs=slicediffs;
run;


proc mixed data=rwa;
class bt rep line;
model lr= bt|line/ddfm=kr;
random rep rep*bt;
lsmeans bt*line;
slice bt*line/ sliceby=line;
slice bt*line/ sliceby=bt;
ods output lsmeans=lsmeans;
```

```
run;
```

SAS input 2011 isolates

Screening of Biotype **1**, Biotype **2** over **34** colonies of unknown Biotypes
Bt: **101**= Biotype **1**, **102**= Biotype **2**, **1**= unknown Biotype colony # **M1**, **2**= unknown Biotype colony #**M2**, **3**= unknown Biotype colony #**M3**, **5**= unknown Biotype colony #**P5**, **6**= unknown Biotype colony #**P6**, **9**= unknown Biotype colony #**P9**, **10**= unknown Biotype colony #**M10**, **11**= unknown Biotype colony #**M11**, **12**= unknown Biotype colony #**M12**, **13**= unknown Biotype colony #**M13**, **14**= unknown Biotype colony #**P14**, **16**= unknown Biotype colony #**P16**, **17**= unknown Biotype colony #**P17**, **18**= unknown Biotype colony #**P18**, **19**= unknown Biotype colony #**P19**, **20**= unknown Biotype colony #**P20**, **21**= unknown Biotype colony #**P21**, **22**= unknown Biotype colony #**P22**, **23**= unknown Biotype colony #**P23**, **24**= unknown Biotype colony #**P24**, **25**= unknown Biotype colony #**P25**, **26**= unknown Biotype colony #**M26**, **27**= unknown Biotype colony #**M27**, **28**= unknown Biotype colony #**M28**, **29**= unknown Biotype colony #**M29**, **30**= unknown Biotype colony #**M30**, **31**= unknown Biotype colony #**M31**, **32**= unknown Biotype colony #**M32**, **33**= unknown Biotype colony #**M33**, **36**= unknown Biotype colony #**M36**, **37**= unknown Biotype colony #**M37**, **38**= unknown Biotype colony #**M38**, **39**= unknown Biotype colony #**M39**, **40**= unknown Biotype colony #**M40**

Rep: repetitions **1**-**6**
Line: plant entries **1**-**8**
Ls: plant chlorosis rating
Lr: leaf rolling rating

```
data rwa;
input bt rep line ls lr;
datalines;
101    1     1     4           2
101    1     4     4           2
101    1     6     3           2.25
101    1     14    2.4         2
101    1     19    7.6         3
101    1     20    3           2
101    1     21    8.25        3
101    1     23    8.2         3
101    2     1     5           3
101    2     4     5.5         2.25
101    2     6     7           2.6
101    2     14    4           2
101    2     19    9           3
101    2     20    4           2
101    2     21    9           3
101    2     23    7           3
101    3     1     7.5         2.25
101    3     4     7.2         3
101    3     6     6.8         3
```

101	3	14	4	2
101	3	19	9	3
101	3	20	4	2
101	3	21	8.25	2.75
101	3	23	8.6	3
101	4	1	4	2
101	4	4	4	2
101	4	6	4.8	2.2
101	4	14	3	2
101	4	19	8	3
101	4	20	3	2
101	4	21	5.8	2.4
101	4	23	7	3
101	5	1	4.75	2
101	5	4	4	2
101	5	6	3.67	2
101	5	14	3.4	2
101	5	19	8.2	3
101	5	20	3.6	2
101	5	21	5	2.33
101	5	23	6.4	2.6
101	6	1	7.2	2.2
101	6	4	4.75	2.75
101	6	6	4.33	2.33
101	6	14	5.25	2.25
101	6	19	8.8	3
101	6	20	4.6	2
101	6	21	6	2.33
101	6	23	8.6	3
102	1	1	9	3
102	1	4	8.75	3
102	1	6	8.4	3
102	1	14	6.8	2.2
102	1	19	8.75	3
102	1	20	6	2
102	1	21	8.6	3
102	1	23	8.5	3
102	2	1	8	2.33
102	2	4	8.25	3
102	2	6	8.4	3
102	2	14	5.2	2
102	2	19	8.5	3
102	2	20	5.5	2
102	2	21	8.5	3
102	2	23	6.4	2.8
102	3	1	7	2.25

102	3	4	9	3
102	3	6	8.75	3
102	3	14	6.6	2.6
102	3	19	9	3
102	3	20	4	2
102	3	21	7.25	2.75
102	3	23	7	3
102	4	1	7	2.2
102	4	4	7.6	3
102	4	6	7.2	2.4
102	4	14	5.75	2.25
102	4	19	9	3
102	4	20	4.6	2.2
102	4	21	7.75	2.75
102	4	23	7.8	3
102	5	1	5	2
102	5	4	8.5	3
102	5	6	8	3
102	5	14	2	1.8
102	5	19	8.33	3
102	5	20	3.6	2
102	5	21	6.75	2.75
102	5	23	7.2	3
102	6	1	6	2
102	6	4	9	3
102	6	6	8.8	3
102	6	14	5.75	2.5
102	6	19	8.6	3
102	6	20	2.5	2
102	6	21	4.6	2.2
102	6	23	4	2
1	1	1	7.8	3
1	1	4	9	3
1	1	6	9	3
1	1	14	6.25	2.25
1	1	19	9	3
1	1	20	6.6	2.2
1	1	21	9	3
1	1	23	6.6	3
1	2	1	7.5	2.25
1	2	4	9	3
1	2	6	9	3
1	2	14	6.8	2.2
1	2	19	9	3
1	2	20	6	2
1	2	21	7	2.6

1	2	23	6.5		3
1	3	1	8.6		2.6
1	3	4	9		3
1	3	6	9		3
1	3	14	7.8		2.6
1	3	19	9		3
1	3	20	4		2
1	3	21	9		3
1	3	23	8.75		3
1	4	1	8		2.25
1	4	4	7.75		3
1	4	6	9		3
1	4	14	5		2
1	4	19	9		3
1	4	20	5		2
1	4	21	8.8		3
1	4	23	7		3
1	5	1	8		2.5
1	5	4	9		3
1	5	6	9		3
1	5	14	3.8		2
1	5	19	8.8		3
1	5	20	3.8		2
1	5	21	7		2.6
1	5	23	8.6		3
1	6	1	5		2.2
1	6	4	8.2		3
1	6	6	8.2		3
1	6	14	8		3
1	6	19	8.6		3
1	6	20	3		2
1	6	21	6.5		3
1	6	23	7		3
2	1	1	6.75		2
2	1	4	8.5		3
2	1	6	8.8		3
2	1	14	3		2
2	1	19	8.5		3
2	1	20	4.6		2
2	1	21	6.8		2.8
2	1	23	5.25		2.5
2	2	1	8.25		2.5
2	2	4	9		3
2	2	6	9		3
2	2	14	8.33	3	
2	2	19	9		3

2	2	20	6.6		2
2	2	21	9		3
2	2	23	7.5		3
2	3	1	8		2.5
2	3	4	9		3
2	3	6	9		5
2	3	14	3.8		2
2	3	19	9		3
2	3	20	1.6		1.6
2	3	21	7.75	2.75	
2	3	23	6.25	2.75	
2	4	1	8		2.4
2	4	4	9		3
2	4	6	9		3
2	4	14	7.5		2.75
2	4	19	8.8		3
2	4	20	3.6		2
2	4	21	7.2		2.6
2	4	23	6.6		3
2	5	1	8.5		2.5
2	5	4	9		3
2	5	6	9		3
2	5	14	4.5		2
2	5	19	8.5		3
2	5	20	3.4		2
2	5	21	7.6		3
2	5	23	7.6		3
2	6	1	6.75	2	
2	6	4	7.2		3
2	6	6	7.4		3
2	6	14	4.5		2
2	6	19	8.6		3
2	6	20	4		2
2	6	21	7.5		2.75
2	6	23	5		3
3	1	1	4		2
3	1	4	8.33	3	
3	1	6	8.8		3
3	1	14	7		2.6
3	1	19	8.75	3	
3	1	20	4.6		2
3	1	21	7.6		2.8
3	1	23	8.2		3
3	2	1	8.33	2.67	
3	2	4	8.75	2.75	
3	2	6	9		3

3	2	14	7.4		2.4
3	2	19	8.5		3
3	2	20	6		2
3	2	21	6.75	2.5	
3	2	23	6.8		3
3	3	1	8.4		2.4
3	3	4	9		3
3	3	6	9		3
3	3	14	6.5		2.75
3	3	19	9		3
3	3	20	3.4		2
3	3	21	7.4		2.8
3	3	23	8.2		3
3	4	1	8.6		2.8
3	4	4	8.2		3
3	4	6	9		3
3	4	14	5		2
3	4	19	9		3
3	4	20	4.4		2
3	4	21	8.4		3
3	4	23	7		3
3	5	1	7.5		2
3	5	4	7		3
3	5	6	8		3
3	5	14	2.5		2
3	5	19	8.4		3
3	5	20	3.5		2
3	5	21	6.2		2.6
3	5	23	5		2
3	6	1	7.5		2.25
3	6	4	9		3
3	6	6	9		3
3	6	14	4.5		2
3	6	19	5.4		3
3	6	20	2.6		2
3	6	21	6.75		2.5
3	6	23	6.8		3
5	1	1	5		2.33
5	1	4	7.75		3
5	1	6	8		3
5	1	14	5.4		2
5	1	19	8		3
5	1	20	5		2.2
5	1	21	6.75		2.75
5	1	23	8		3
5	2	1	7		2.5

5	2	4	8.25	3
5	2	6	8.8	3
5	2	14	6	2.33
5	2	19	8.5	3
5	2	20	4	2
5	2	21	6.2	2.4
5	2	23	5.75	2.75
5	3	1	9	3
5	3	4	9	3
5	3	6	9	3
5	3	14	9	3
5	3	19	9	3
5	3	20	5	2
5	3	21	9	3
5	3	23	9	3
5	4	1	7.2	2
5	4	4	9	3
5	4	6	9	3
5	4	14	6	2
5	4	19	8.6	3
5	4	20	4.2	2
5	4	21	9	3
5	4	23	7.6	3
5	5	1	7	2
5	5	4	8.75	3
5	5	6	7.8	2
5	5	14	3	2
5	5	19	8.4	3
5	5	20	2.8	2
5	5	21	5	2.25
5	5	23	8.2	3
5	6	1	6	2
5	6	4	7	3
5	6	6	8.6	3
5	6	14	3.25	2
5	6	19	8.8	3
5	6	20	4	2
5	6	21	8	3
5	6	23	6	3
6	1	1	8	2.5
6	1	4	9	3
6	1	6	9	3
6	1	14	4.75	2
6	1	19	8.8	3
6	1	20	5.8	2
6	1	21	9	3

6	1	23	8.6	3
6	2	1	8	2
6	2	4	9	3
6	2	6	8.75	3
6	2	14	5.67	2.33
6	2	19	9	3
6	2	20	4.4	2
6	2	21	8.5	2.75
6	2	23	7.4	3
6	3	1	9	3
6	3	4	9	3
6	3	6	9	3
6	3	14	5.4	2.2
6	3	19	9	3
6	3	20	4.75	2
6	3	21	7.4	2.6
6	3	23	7.4	3
6	4	1	8.2	2.2
6	4	4	8.33	3
6	4	6	9	3
6	4	14	5	2
6	4	19	8.5	3
6	4	20	5.5	2
6	4	21	9	3
6	4	23	7.6	3
6	5	1	5.67	2
6	5	4	5.5	3
6	5	6	9	3
6	5	14	4	2
6	5	19	9	3
6	5	20	3.2	2
6	5	21	5.2	2.5
6	5	23	8	3
6	6	1	6	2
6	6	4	8	3
6	6	6	7.8	3
6	6	14	2	2
6	6	19	8.2	3
6	6	20	3	2
6	6	21	4.67	2.33
6	6	23	8.4	3
9	1	1	6	2.2
9	1	4	9	3
9	1	6	8.8	3
9	1	14	4.4	2
9	1	19	9	3

9	1	20	5	2
9	1	21	8.2	3
9	1	23	6.2	3
9	2	1	8	3
9	2	4	8.8	3
9	2	6	8.8	3
9	2	14	7.25	2.5
9	2	19	8	3
9	2	20	3.5	2
9	2	21	7.33	2.67
9	2	23	7.6	3
9	3	1	9	3
9	3	4	9	3
9	3	6	9	3
9	3	14	9	3
9	3	19	9	3
9	3	20	4.4	2
9	3	21	9	3
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9	4	4	7.6	3
9	4	6	9	3
9	4	14	4.6	2.2
9	4	19	8.75	3
9	4	20	4.4	2
9	4	21	7	2.6
9	4	23	7.5	3
9	5	1	7.4	2
9	5	4	8.4	3
9	5	6	8.4	3
9	5	14	3	2
9	5	19	8.6	3
9	5	20	3.2	2
9	5	21	8.6	3
9	5	23	8	3
9	6	1	5.67	2
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9	6	20	2.4	2
9	6	21	6	2.6
9	6	23	6	3
10	1	1	7.75	2.25
10	1	4	8	3
10	1	6	3.25	2

10	1	14	7	2.33
10	1	19	8.75	3
10	1	20	3.4	2
10	1	21	8	3
10	1	23	7.5	3
10	2	1	6.5	2
10	2	4	6.75	3
10	2	6	6.5	2.75
10	2	14	6.25	2.25
10	2	19	8.5	3
10	2	20	3.4	2
10	2	21	5.6	2.4
10	2	23	6.8	3
10	3	1	7.75	2.25
10	3	4	6.4	3
10	3	6	3.4	2
10	3	14	4.67	2
10	3	19	8.4	3
10	3	20	3.8	2
10	3	21	6.5	3
10	3	23	6.2	3
10	4	1	8.75	2.75
10	4	4	7.25	3
10	4	6	3.75	2
10	4	14	4.5	2
10	4	19	9	3
10	4	20	4	2
10	4	21	7.2	2.8
10	4	23	7.5	3
10	5	1	7.25	2
10	5	4	7.4	3
10	5	6	4	2
10	5	14	3.5	2
10	5	19	9	3
10	5	20	3.25	2
10	5	21	6.33	2.33
10	5	23	7.25	3
10	6	1	7.5	2.5
10	6	4	7.2	3
10	6	6	3.5	2
10	6	14	6.5	2.25
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10	6	20	4.6	2
10	6	21	6.25	2.5
10	6	23	7	3
11	1	1	4.33	2

11	1	4	7.75	3
11	1	6	8	3
11	1	14	5	1
11	1	19	8.67	3
11	1	20	4	2
11	1	21	5.75	2.5
11	1	23	6.8	2.2
11	2	1	4	2
11	2	4	9	3
11	2	6	8	3
11	2	14	4.4	2
11	2	19	9	3
11	2	20	3.4	2
11	2	21	7	2.75
11	2	23	7.2	3
11	3	1	8.2	2.4
11	3	4	8.75	3
11	3	6	9	3
11	3	14	3.6	2
11	3	19	9	3
11	3	20	3.2	2
11	3	21	7.67	2.67
11	3	23	7.2	3
11	4	1	7.4	2.2
11	4	4	7.33	3
11	4	6	9	3
11	4	14	5.33	2
11	4	19	7.2	2.4
11	4	20	4	2
11	4	21	7	2.6
11	4	23	6	3
11	5	1	6.6	2
11	5	4	9	3
11	5	6	8	3
11	5	14	3.5	2
11	5	19	8.67	3
11	5	20	3	1.8
11	5	21	6	2.6
11	5	23	8	3
11	6	1	6.6	2.4
11	6	4	9	3
11	6	6	9	3
11	6	14	2.6	2
11	6	19	8.4	3
11	6	20	3	2
11	6	21	3.75	2.25

11	6	23	8	3
12	1	1	7.67	2.67
12	1	4	7	3
12	1	6	6.4	2.6
12	1	14	5.2	2
12	1	19	9	3
12	1	20	3.4	2
12	1	21	7.8	2.8
12	1	23	6.25	2.5
12	2	1	6.75	2.25
12	2	4	7.5	3
12	2	6	5.5	2.25
12	2	14	6.4	2
12	2	19	8	3
12	2	20	4	2
12	2	21	7.75	2.75
12	2	23	8.4	3
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26	5	20	5.4	2
26	5	21	3.33	2
26	5	23	8	3
26	6	1	8.75	2.75
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26	6	6	6.8	3
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26	6	23	8.4	3
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27	1	6	4.8	2.8
27	1	14	6.33	2
27	1	19	8.6	3
27	1	20	4.2	2
27	1	21	8	2.8
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27	2	4	7.6	3
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27	5	19	8.6	3
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27	5	21	6.75	2.5
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27	6	4	8.2	3
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27	6	21	7	3
27	6	23	7.75	3
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37	6	19	8.6	3
37	6	20	4	2
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38	6	6	6.2	2.4
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38	6	19	9	3

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39	1	14	7.4	3
39	1	19	8.6	3
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40	5	6	3.6	2
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40	5	19	9	3
40	5	20	2.6	2
40	5	21	7	3
40	5	23	6.4	3
40	6	1	8.2	2.6

```
40      6       4       4.6             2.6
40      6       6       4.8             2
40      6       14      5.5             2.25
40      6       19      9               3
40      6       20      5.2             2
40      6       21      7.2             2.8
40      6       23      9               3
```

```
proc mixed data=rwa;
class bt rep line;
model ls= bt|line/ddfm=kr residual;
random rep rep*bt;
lsmeans bt*line;
slice bt*line/ sliceby=line pdiff;
ods output slicediffs=slicediffs;
run;
```

```
proc mixed data=rwa;
class bt rep line;
model lr= bt|line/ddfm=kr;
random rep rep*bt;
lsmeans bt*line;
slice bt*line/ sliceby=line;
slice bt*line/ sliceby=bt;
ods output lsmeans=lsmeans;
run;
```

APPENDIX II. R input

2010 isolates: leaf chlorosis

#Read in the data (Screenings2010wnames.csv is the table with the mean values of leaf chlorosis of 2010 isolates biotyped with the 24 set of differentials, obtained with the PROC MIXED procedure (SAS))

Screenings2010wnames.csv

	RWA1	M 2	M 3	M 4	M 5	P 6	P 7	M 8	M 9	M 10	M 11	M 12	M 13	P 14	P 15	RWA2
CO03797	6.57	4.63	5.85	6.48	7,93	6.02	4.85	5.73	5.53	5.90	6.48	6.77	6.84	7.21	6.35	6.14
CO03804	6.53	6.12	7.28	7.40	7,31	7.59	7.91	5.64	5.55	6.74	8.15	6.95	6.59	8.09	6.94	7.14
CO03811	6.48	7.05	7.42	7.66	8,53	7.38	7.86	6.90	6.77	7.73	7.95	7.56	7.52	8.67	6.85	8.01
Yumar	5.73	6.45	6.29	6.73	7,02	7.19	7.78	5.61	5.09	7.51	7.05	5.83	6.44	8.69	6.43	7.95
CO950043	5.17	5.55	7.08	6.45	7,62	7.47	7.93	5.88	5.48	6.44	6.93	6.84	6.81	8.77	6.88	8.31
CO960223	4.48	3.73	3.59	3.75	5,92	6.70	7.11	5.87	3.55	4.34	4.02	4.88	5.11	8.60	4.99	7.72
94M370	4.38	3.08	3.00	3.86	5,20	3.14	3.05	4.28	3.89	3.53	3.68	3.71	4.63	4.74	3.45	3.17
Karee-Dn8	7.16	4.63	5.78	7.53	7,94	5.58	5.34	6.54	6.23	6.54	5.21	6.47	7.49	7.17	6.59	4.60
Betta-Dn9	6.23	5.06	5.68	6.01	7,21	5.95	6.05	7.06	6.58	5.57	5.55	6.22	6.87	5.94	5.58	4.98
KS94WGRC29	4.58	3.15	3.25	3.97	4,88	8.00	8.21	4.25	3.97	3.58	3.83	3.28	5.21	8.33	4.43	7.97
Stanton	6.18	7.16	7.44	7.73	8,21	8.64	8.17	7.13	5.27	7.65	7.80	7.26	7.31	8.75	7.11	8.41
STARS 9302W	6.20	4.28	4.09	4.88	5,47	6.08	5.52	5.79	5.70	4.48	4.71	5.18	5.62	6.53	4.33	5.13
KS92WGRC25	7.38	5.98	6.25	6.56	7,57	6.76	6.61	6.83	6.17	6.20	5.91	7.36	6.69	6.97	6.77	6.38
CI2401	4.23	3.90	5.57	4.10	5,46	4.20	4.17	4.30	4.19	4.73	5.49	4.78	4.63	5.65	3.89	4.04
PI626197	4.74	4.64	5.00	5.69	5,87	4.43	4.29	5.81	4.99	5.07	5.60	5.14	5.45	5.41	5.11	5.15
PI625139	5.87	5.87	5.18	5.98	6,38	6.28	5.61	7.82	5.98	5.13	6.22	5.77	5.98	6.33	5.35	5.23
PI626580-4	4.13	3.75	3.96	4.03	5,48	3.10	3.93	3.95	4.03	4.02	4.27	4.73	4.74	4.18	3.31	5.37
Hatcher	6.25	6.18	6.20	6.89	7,63	7.53	7.87	5.68	5.27	7.27	7.20	6.40	6.72	8.83	6.62	7.73
Yuma	8.04	7.60	8.11	8.42	8,51	8.20	7.42	8.00	7.59	8.51	8.27	8.44	8.55	8.92	7.77	7.87
CO08RWA50	3.55	2.77	3.47	3.40	4,13	2.70	3.50	4.34	3.90	3.72	3.50	3.41	3.40	3.46	3.17	3.27
CO03765	5.45	4.19	6.05	5.93	5,68	5.22	5.27	4.35	5.73	6.13	6.35	6.02	5.11	6.03	6.07	6.70
Otis	8.47	8.57	7.65	8.17	8,60	7.93	8.23	8.23	8.37	8.07	8.50	8.43	8.31	8.32	8.16	7.48
Stoneham	5.30	4.86	5.17	5.17	6,77	5.32	4.87	6.35	5.50	4.42	5.06	5.17	5.67	5.26	5.34	5.40
Sidney	5.48	4.10	3.78	4.93	5,23	3.00	4.48	5.68	4.07	4.49	4.16	3.92	4.73	4.48	4.73	3.37

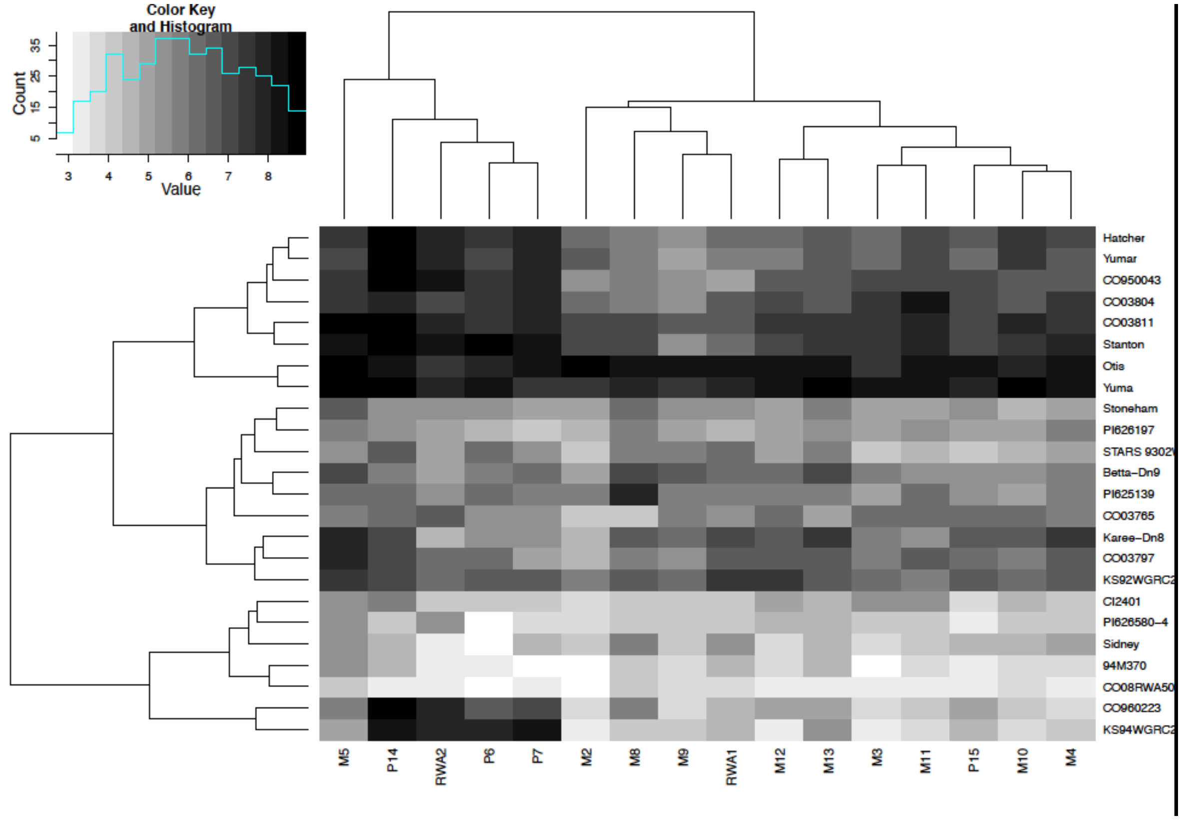
```
Screen2010<-read.csv("c:/Documents and
Settings/insectaryusr/Desktop/Mariana/Hess/Screenings2010wnames.csv",header=T)
#Drop "extra" empty rows
Screen2010<-Screen2010[1:24,]
row.names(Screen2010)<-Screen2010[,1]
Screen2010<-Screen2010[,-1]
Screen2010<-as.matrix(Screen2010)
```

```
#Heatmap

library(gplots)

heatmap.2(Screen2010,col=colorRampPalette(c("white","black"))(15),trace="none")
```

Heatmap output for leaf chlorosis:



```
#Correlation

cor(Screen2010)
```

Correlation output for leaf chlorosis:

	RWA1	M 2	M 3	M 4	M 5	P 6	P 7	M 8	M 9	M 10	M 11	M 12	M 13	P 14	P 15	RWA2
RWA1	1.000	0.808	0.736	0.875	0.832	0.626	0.531	0.798	0.892	0.789	0.721	0.863	0.897	0.555	0.852	0.401
M 2	0.808	1.000	0.889	0.901	0.867	0.731	0.703	0.807	0.808	0.913	0.920	0.894	0.874	0.667	0.882	0.629
M 3	0.736	0.889	1.000	0.919	0.880	0.683	0.631	0.635	0.777	0.940	0.961	0.936	0.847	0.645	0.904	0.619
M 4	0.875	0.901	0.919	1.000	0.922	0.692	0.627	0.743	0.835	0.950	0.908	0.926	0.933	0.657	0.947	0.564
M 5	0.832	0.867	0.880	0.922	1.000	0.715	0.633	0.761	0.786	0.886	0.843	0.938	0.959	0.703	0.917	0.604
P 6	0.626	0.731	0.683	0.692	0.715	1.000	0.945	0.586	0.552	0.711	0.721	0.694	0.774	0.944	0.792	0.893
P 7	0.531	0.703	0.631	0.627	0.633	0.945	1.000	0.489	0.449	0.689	0.675	0.607	0.684	0.925	0.746	0.910
M 8	0.798	0.807	0.635	0.743	0.761	0.586	0.489	1.000	0.806	0.651	0.620	0.753	0.805	0.474	0.732	0.347
M 9	0.892	0.808	0.777	0.835	0.786	0.552	0.449	0.806	1.000	0.759	0.745	0.863	0.855	0.435	0.790	0.360
M 10	0.789	0.912	0.940	0.950	0.886	0.711	0.689	0.651	0.759	1.000	0.936	0.913	0.879	0.725	0.928	0.660
M 11	0.721	0.920	0.961	0.908	0.843	0.721	0.675	0.620	0.745	0.936	1.000	0.901	0.822	0.688	0.888	0.667
M 12	0.863	0.894	0.936	0.926	0.938	0.694	0.607	0.753	0.863	0.913	0.901	1.000	0.920	0.651	0.923	0.600
M 13	0.897	0.874	0.847	0.933	0.959	0.774	0.684	0.805	0.855	0.879	0.822	0.920	1.000	0.740	0.916	0.614
P 14	0.555	0.667	0.645	0.657	0.703	0.944	0.925	0.474	0.435	0.725	0.688	0.651	0.740	1.000	0.769	0.902
P 15	0.852	0.882	0.904	0.947	0.917	0.792	0.746	0.732	0.790	0.928	0.888	0.923	0.916	0.769	1.000	0.702
RWA2	0.401	0.629	0.619	0.564	0.604	0.893	0.910	0.347	0.360	0.660	0.667	0.600	0.614	0.901	0.702	1.000

```
#Calculate Means and Standard Deviations

apply(Screen2010,2,mean)

apply(Screen2010,2,sd)
```

Means and Standard Deviations outputs for leaf chlorosis:

	RWA1	M 2	M 3	M 4	M 5	P 6	P 7	M 8	M 9	M 10	M 11	M 12	M 13	P 14	P 15	RWA2
Mean	5.774	5.138	5.548	5.905	6.690	6.017	6.085	5.918	5.392	5.740	5.912	5.855	6.100	6.889	5.676	6.147
SD	1.257	1.526	1.526	1.528	1.320	1.799	1.710	1.246	1.219	1.532	1.576	1.475	1.285	1.719	1.421	1.719

2010 isolates: leaf rolling

#Read in the data (Screenings2010rolling.csv is the table with the mean values of leaf rolling of 2010 isolates biotyped with the 24 set of differentials, obtained with the PROC MIXED procedure (SAS))

Screenings2010rolling.csv

	RWA1	M 2	M 3	M 4	M 5	P 6	P 7	M 8	M 9	M 10	M 11	M 12	M 13	P 14	P 15	RWA2
CO03797	2.34	2.08	2.01	2.10	2.56	1.76	1.88	2.27	2.31	2.17	2.19	2.30	2.32	2.42	2.30	2.17
CO03804	2.78	2.83	2.88	2.97	2.86	3.00	3.00	2.39	2.49	2.79	2.93	2.87	2.87	2.90	3.00	2.86
CO03811	2.63	2.87	2.83	2.97	3.00	2.93	3.00	2.88	2.75	3.00	2.97	3.00	3.00	3.00	2.93	3.00
Yumar	2.37	2.77	3.67	3.00	2.95	2.90	2.96	2.51	2.29	3.00	2.80	2.77	2.83	3.00	2.92	3.00
CO950043	2.03	2.56	2.62	2.69	2.72	2.83	2.87	2.30	1.97	2.47	2.90	2.77	2.82	3.00	2.53	2.96
CO960223	1.92	2.05	2.22	1.95	2.43	2.77	2.93	2.48	1.80	2.10	1.75	2.27	2.38	3.00	2.18	2.96
94M370	1.38	1.60	1.43	1.56	2.20	1.38	1.33	1.57	1.63	1.49	1.50	1.57	1.73	1.73	1.52	1.33
Karee-Dn8	2.30	1.70	2.29	2.3	2.70	2.04	1.83	2.40	2.32	2.42	1.95	2.47	2.5	2.50	2.31	1.88
Betta-Dn9	1.80	1.93	1.77	1.95	2.27	1.75	1.83	2.33	2.20	1.93	1.89	2.01	2.3	2.13	1.80	1.91
KS94WGRC29	2.25	1.40	1.50	2.07	1.91	2.90	3.00	1.87	1.63	1.78	1.79	1.54	2.07	2.92	2.13	3.00
Stanton	2.46	2.80	2.97	3.00	3.00	3.00	2.88	2.64	2.23	3.00	3.00	3.00	2.97	3.00	2.97	3.00
STARS 9302W	2.26	2.01	1.73	1.67	2.10	2.57	2.05	2.08	2.06	1.83	1.73	2.00	2.05	2.36	1.73	2.07
KS92WGRC25	2.55	2.15	2.25	2.13	2.45	2.16	2.16	2.30	2.09	2.12	1.73	2.49	2.24	2.38	2.28	2.17
CI2401	1.95	1.87	2.10	1.83	2.07	1.75	1.93	2.00	2.00	1.97	1.82	1.99	1.94	2.13	2.10	1.89
PI626197	1.89	2.10	1.90	2.00	2.30	1.68	1.88	2.02	1.92	2.07	2.03	1.94	2.05	2.23	2.23	1.97
PI625139	2.03	2.38	2.06	1.93	2.57	2.32	2.33	2.57	2.17	2.13	2.22	2.36	2.4	2.57	2.50	2.23
PI626580-4	1.69	1.53	1.67	1.40	2.03	1.67	1.77	1.67	1.57	1.67	1.83	1.87	2.04	1.70	1.50	1.83
Hatcher	2.40	2.83	2.80	2.96	2.93	2.83	2.97	2.34	2.17	2.83	3.00	2.83	2.9	3.00	2.92	3.00
Yuma	3.00	2.83	3.00	3.00	3.00	3.00	2.93	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	2.95
CO08RWA50	1.62	1.60	1.83	1.83	1.67	1.03	1.53	1.80	1.80	1.87	1.75	1.73	1.77	1.68	1.33	1.40
CO03765	2.40	1.98	2.60	2.40	2.47	2.20	2.28	2.14	2.31	2.81	2.57	2.45	2.39	2.47	2.58	2.75
Otis	3.00	3.00	3.00	2.67	3.00	3.00	2.90	2.83	3.00	3.00	2.87	2.97	3.00	2.88	2.96	2.86
Stoneham	2.03	2.17	1.83	2.07	2.63	2.00	2.07	2.30	2.00	2.04	2.17	2.30	2.17	2.17	2.17	2.17
Sidney	1.73	2.03	1.72	1.83	1.90	1.50	1.58	2.05	1.83	1.97	1.91	1.77	2.17	1.60	1.80	1.60

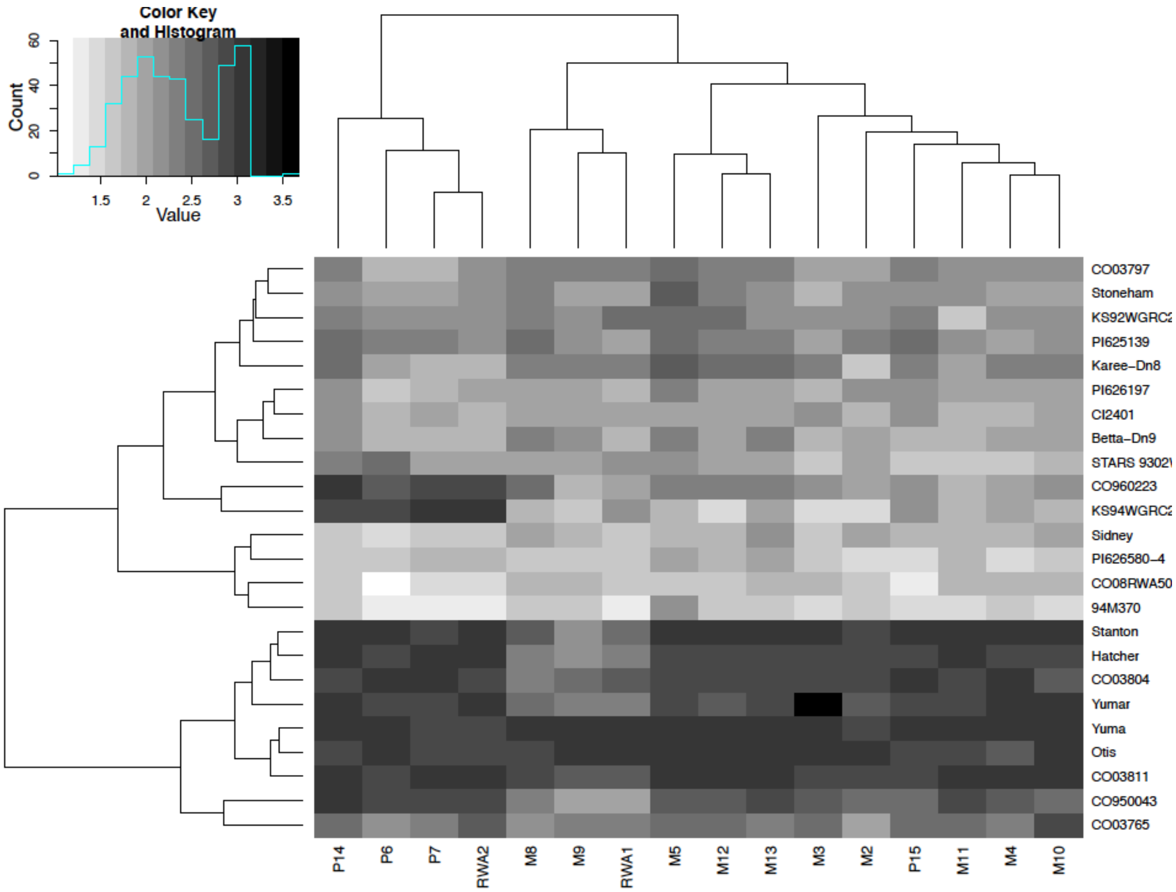
```
Screen2010rolling<-read.csv("c:/Documents and Settings/insectaryusr/Desktop/Mariana lab/Hess/Screenings2010rolling.csv",header=T)
#Drop "extra" empty rows
Screen2010rolling<-Screen2010rolling[1:24,]
row.names(Screen2010rolling)<-Screen2010rolling[,1]
Screen2010rolling<-Screen2010rolling[,-1]
Screen2010rolling<-as.matrix(Screen2010rolling)
```

```
#Heatmap

library(gplots)

heatmap.2(Screen2010rolling,col=colorRampPalette(c("white","black"))(15),trace="none")
```

Heatmap output for leaf rolling:



```
#Correlation
```

```
cor(Screen2010rolling)
```

Correlation output for leaf rolling:

```
RWA1      M.2      M.3      M.4      M.5      P.6      P.7
RWA1 1.0000000 0.7206963 0.7331739 0.7755373 0.7423994 0.7652134 0.7128768
M.2  0.7206963 1.0000000 0.8516369 0.8569904 0.8693114 0.7204717 0.7190131
M.3  0.7331739 0.8516369 1.0000000 0.9096640 0.8406131 0.7104586 0.7352061
M.4  0.7755373 0.8569904 0.9096640 1.0000000 0.8607804 0.7672902 0.7999936
M.5  0.7423994 0.8693114 0.8406131 0.8607804 1.0000000 0.7267756 0.6908289
P.6  0.7652134 0.7204717 0.7104586 0.7672902 0.7267756 1.0000000 0.9577634
P.7  0.7128768 0.7190131 0.7352061 0.7999936 0.6908289 0.9577634 1.0000000
M.8  0.7727624 0.8230546 0.7467184 0.7550664 0.8364822 0.7049491 0.6808155
M.9  0.8559756 0.7591174 0.7206781 0.7226839 0.7658860 0.5474235 0.4988662
M.10 0.8097860 0.8647439 0.9485350 0.9469222 0.8696184 0.7204559 0.7359082
M.11 0.7139111 0.8958144 0.8677708 0.9312224 0.8484109 0.7189128 0.7496808
M.12 0.8076731 0.9064194 0.9046591 0.8965127 0.9425593 0.7447358 0.7288012
M.13 0.7856801 0.8987406 0.8832155 0.9253019 0.9103098 0.8208285 0.8127179
P.14 0.7400452 0.7000455 0.7417300 0.8120888 0.7700413 0.9410496 0.9461562
P.15 0.8394451 0.8754586 0.8810543 0.9246233 0.9093395 0.8167882 0.8257612
RWA2 0.7189933 0.6926876 0.7405413 0.8040951 0.7100241 0.9379441 0.9759643
          M.8      M.9     M.10     M.11     M.12     M.13     P.14
RWA1 0.7727624 0.8559756 0.8097860 0.7139111 0.8076731 0.7856801 0.7400452
M.2  0.8230546 0.7591174 0.8647439 0.8958144 0.9064194 0.8987406 0.7000455
M.3  0.7467184 0.7206781 0.9485350 0.8677708 0.9046591 0.8832155 0.7417300
M.4  0.7550664 0.7226839 0.9469222 0.9312224 0.8965127 0.9253019 0.8120888
M.5  0.8364822 0.7658860 0.8696184 0.8484109 0.9425593 0.9103098 0.7700413
P.6  0.7049491 0.5474235 0.7204559 0.7189128 0.7447358 0.8208285 0.9410496
```

```
P.7  0.6808155 0.4988662 0.7359082 0.7496808 0.7288012 0.8127179 0.9461562
M.8  1.0000000 0.8580141 0.8079062 0.7211530 0.8648834 0.8624479 0.7382313
M.9  0.8580141 1.0000000 0.8139936 0.7206323 0.8044910 0.7752974 0.5607515
M.10 0.8079062 0.8139936 1.0000000 0.9281582 0.9293081 0.9285192 0.7520494
M.11 0.7211530 0.7206323 0.9281582 1.0000000 0.8941153 0.9248025 0.7237112
M.12 0.8648834 0.8044910 0.9293081 0.8941153 1.0000000 0.9460185 0.7683156
M.13 0.8624479 0.7752974 0.9285192 0.9248025 0.9460185 1.0000000 0.8234125
P.14 0.7382313 0.5607515 0.7520494 0.7237112 0.7683156 0.8234125 1.0000000
P.15 0.8188852 0.7619436 0.9279966 0.8950060 0.9096092 0.9161974 0.8586200
RWA2 0.6689183 0.5027739 0.7639760 0.7688870 0.7385482 0.8138054 0.9455985
          P.15      RWA2
RWA1 0.8394451 0.7189933
M.2  0.8754586 0.6926876
M.3  0.8810543 0.7405413
M.4  0.9246233 0.8040951
M.5  0.9093395 0.7100241
P.6  0.8167882 0.9379441
P.7  0.8257612 0.9759643
M.8  0.8188852 0.6689183
M.9  0.7619436 0.5027739
M.10 0.9279966 0.7639760
M.11 0.8950060 0.7688870
M.12 0.9096092 0.7385482
M.13 0.9161974 0.8138054
P.14 0.8586200 0.9455985
P.15 1.0000000 0.8401508
RWA2 0.8401508 1.0000000
```

```
#Calculate Means and Standard Deviations


apply(Screen2010rolling,2,mean)

apply(Screen2010rolling,2,sd)
```

Means and Standard Deviations outputs for leaf rolling:

	RWA1	M 2	M 3	M 4	M 5	P 6	P 7	M 8	M 9	M 10	M 11	M 12	M 13	P 14	P 15	RWA2
Mean	2.200	2.211	2.278	2.262	2.488	2.290	2.329	2.281	2.148	2.311	2.263	2.345	2.413	2.490	2.320	2.373
SD	0.423	0.490	0.585	0.516	0.405	0.622	0.573	0.364	0.384	0.493	0.529	0.481	0.414	0.487	0.524	0.576

2010 isolates: plant differentials

#Read in the data (Diff24b.csv is the table with the mean values of leaf chlorosis of 2010 isolates biotyped with the 24 set of differentials, obtained with the PROC MIXED procedure (SAS))

Diff24b.csv

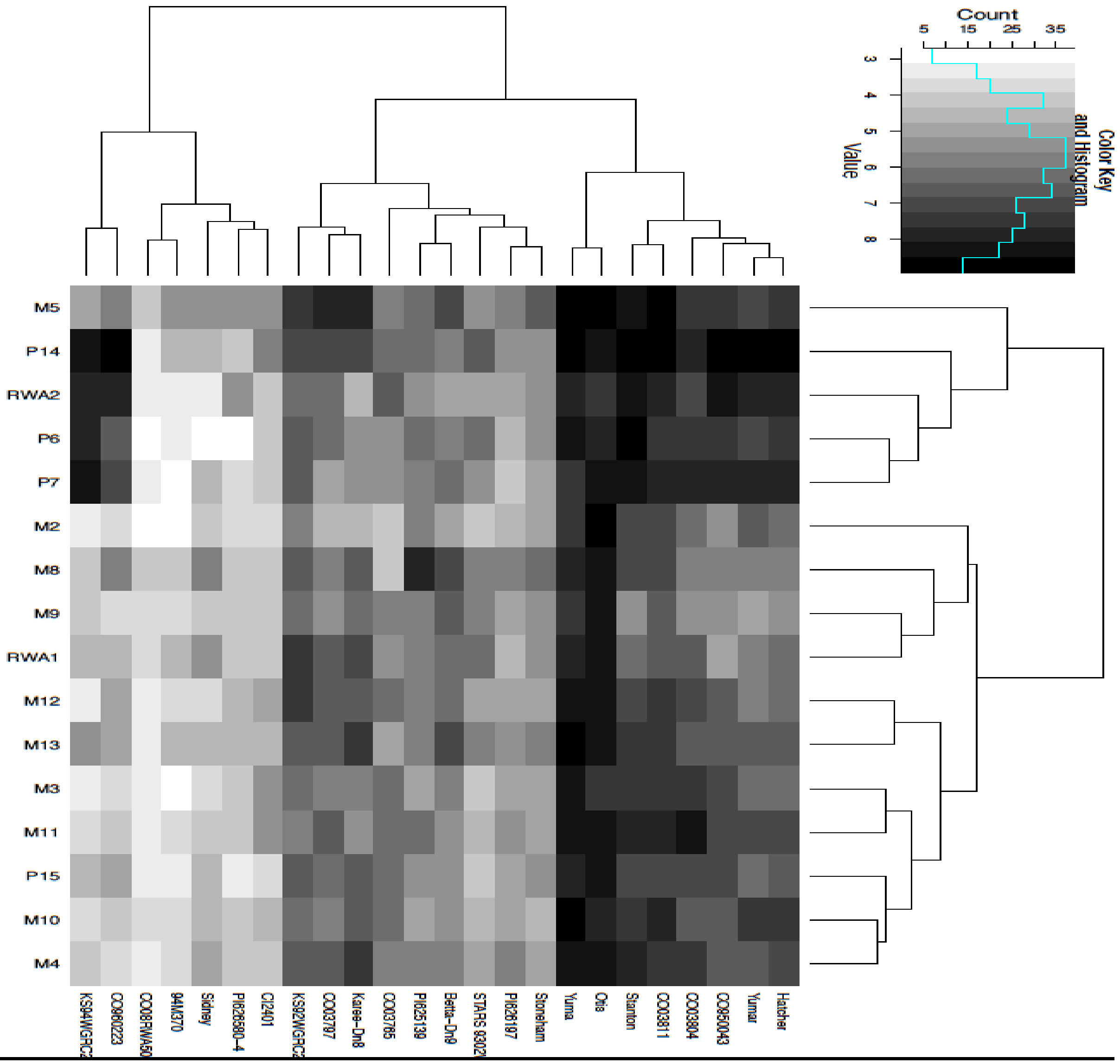
	CO03797	CO03804	CO03811	Yumar	CO950043	CO960223	94M370	Karee-Dn8	Betta-Dn9	KS94WGR C29	Stanton	STARS 9302W	KS92WGR C25	CI2401	PI626197	PI625139	PI626580-4	Hatcher	Yuma	CO08RWA 50	CO03765	Otis	Stoneham	Sidney
RWA1	6.57	6.53	6.48	5.73	5.17	4.48	4.38	7.16	6.23	4.58	6.18	6.20	7.38	4.23	4.74	5.87	4.13	6.25	8.04	3.55	5.45	8.47	5.30	5.48
M 2	4.63	6.12	7.05	6.45	5.55	3.73	3.08	4.63	5.06	3.15	7.16	4.28	5.98	3.90	4.64	5.87	3.75	6.18	7.60	2.77	4.19	8.57	4.86	4.10
M 3	5.85	7.28	7.42	6.29	7.08	3.59	3.00	5.78	5.68	3.25	7.44	4.09	6.25	5.57	5.00	5.18	3.96	6.20	8.11	3.47	6.05	7.65	5.17	3.78
M 4	6.48	7.40	7.66	6.73	6.45	3.75	3.86	7.53	6.01	3.97	7.73	4.88	6.56	4.10	5.69	5.98	4.03	6.89	8.42	3.40	5.93	8.17	5.17	4.93
M 5	7.93	7.31	8.53	7.02	7.62	5.92	5.20	7.94	7.21	4.88	8.21	5.47	7.57	5.46	5.87	6.38	5.48	7.63	8.51	4.13	5.68	8.60	6.77	5.23
P 6	6.02	7.59	7.38	7.19	7.47	6.70	3.14	5.58	5.95	8.00	8.64	6.08	6.76	4.20	4.43	6.28	3.10	7.53	8.20	2.70	5.22	7.93	5.32	3.00
P 7	4.85	7.91	7.86	7.78	7.93	7.11	3.05	5.34	6.05	8.21	8.17	5.52	6.61	4.17	4.29	5.61	3.93	7.87	7.42	3.50	5.27	8.23	4.87	4.48
M 8	5.73	5.64	6.90	5.61	5.88	5.87	4.28	6.54	7.06	4.25	7.13	5.79	6.83	4.30	5.81	7.82	3.95	5.68	8.00	4.34	4.35	8.23	6.35	5.68
M 9	5.53	5.55	6.77	5.09	5.48	3.55	3.89	6.23	6.58	3.97	5.27	5.70	6.17	4.19	4.99	5.98	4.03	5.27	7.59	3.90	5.73	8.37	5.50	4.07
M 10	5.90	6.74	7.73	7.51	6.44	4.34	3.53	6.54	5.57	3.58	7.65	4.48	6.20	4.73	5.07	5.13	4.02	7.27	8.51	3.72	6.13	8.07	4.42	4.49
M 11	6.48	8.15	7.95	7.05	6.93	4.02	3.68	5.21	5.55	3.83	7.80	4.71	5.91	5.49	5.60	6.22	4.27	7.20	8.27	3.50	6.35	8.50	5.06	4.16
M 12	6.77	6.95	7.56	5.83	6.84	4.88	3.71	6.47	6.22	3.28	7.26	5.18	7.36	4.78	5.14	5.77	4.73	6.40	8.44	3.41	6.02	8.43	5.17	3.92
M 13	6.84	6.59	7.52	6.44	6.81	5.11	4.63	7.49	6.87	5.21	7.31	5.62	6.69	4.63	5.45	5.98	4.74	6.72	8.55	3.40	5.11	8.31	5.67	4.73
P 14	7.21	8.09	8.67	8.69	8.77	8.60	4.74	7.17	5.94	8.33	8.75	6.53	6.97	5.65	5.41	6.33	4.18	8.83	8.92	3.46	6.03	8.32	5.26	4.48
P 15	6.35	6.94	6.85	6.43	6.88	4.99	3.45	6.59	5.58	4.43	7.11	4.33	6.77	3.89	5.11	5.35	3.31	6.62	7.77	3.17	6.07	8.16	5.34	4.73
RWA2	6.14	7.14	8.01	7.95	8.31	7.72	3.17	4.60	4.98	7.97	8.41	5.13	6.38	4.04	5.15	5.23	5.37	7.73	7.87	3.27	6.70	7.48	5.40	3.37

```
Diff24b<-read.csv("c:/Documents and Settings/insectaryusr/Desktop/Mariana/Hess/Diff24b.csv",header=T)
Diff24b<-Diff24b[,-1]
Diff24b<-as.matrix(Diff24b)
```

```
#Heatmap

library(gplots)

heatmap.2(Diff24b,col=colorRampPalette(c("white","black"))(15),trace="none")
```



```
#Correlation

cor(Diff24b)
```

Correlation output for plant differentials:

	CO03797	CO03804	CO03811	Yumar	CO950043	CO960223	94M370	Karee-Dn8	Betta-Dn9	KS94WG RC29	Stanton	STARS 9302W	KS92WG RC25	CI2401	PI626197
CO03797	1.000	0.331	0.478	0.158	0.341	0.221	0.758	0.717	0.426	0.058	0.256	0.320	0.650	0.559	0.644
CO03804	0.331	1.000	0.719	0.755	0.768	0.428	0.089	0.060	0.289	0.496	0.773	0.025	0.065	0.484	0.024
CO03811	0.478	0.719	1.000	0.801	0.829	0.549	0.257	0.106	0.029	0.447	0.793	0.102	0.109	0.629	0.345
Yumar	0.158	0.755	0.801	1.000	0.833	0.701	0.065	0.156	0.403	0.702	0.867	0.099	0.068	0.286	0.053
CO950043	0.342	0.768	0.829	0.833	1.000	0.786	0.003	0.081	0.163	0.737	0.847	0.168	0.150	0.410	0.057
CO960223	0.221	0.428	0.549	0.701	0.786	1.000	0.164	0.043	0.025	0.908	0.680	0.588	0.367	0.084	0.053
94M370	0.758	0.089	0.257	0.065	0.003	0.163	1.000	0.823	0.744	0.014	0.063	0.561	0.616	0.399	0.664
Karee-Dn8	0.717	0.060	0.106	0.156	0.081	0.043	0.823	1.000	0.711	0.155	0.117	0.357	0.653	0.266	0.547
Betta-Dn9	0.426	0.289	0.029	0.403	0.163	0.025	0.744	0.711	1.000	0.075	0.238	0.539	0.589	0.190	0.434
KS94WGRC29	0.058	0.496	0.447	0.702	0.737	0.908	0.014	0.155	0.075	1.000	0.621	0.595	0.186	0.058	0.291
Stanton	0.256	0.773	0.793	0.867	0.847	0.680	0.063	0.117	0.238	0.621	1.000	0.067	0.099	0.328	0.076
STARS 9302W	0.320	0.025	0.102	0.099	0.168	0.588	0.561	0.357	0.539	0.595	0.067	1.000	0.553	0.038	0.002
KS92WGRC25	0.650	0.065	0.109	0.068	0.150	0.367	0.616	0.653	0.589	0.186	0.099	0.553	1.000	0.123	0.185
CI2401	0.559	0.484	0.629	0.286	0.410	0.084	0.399	0.266	0.190	0.058	0.328	0.038	0.123	1.000	0.435
PI626197	0.645	0.024	0.345	0.053	0.057	0.053	0.664	0.547	0.434	0.291	0.076	0.002	0.185	0.435	1.000
PI625139	0.148	0.235	0.048	0.230	0.163	0.183	0.519	0.263	0.628	0.030	0.008	0.532	0.280	0.075	0.461
PI626580-4	0.535	0.061	0.525	0.148	0.297	0.210	0.471	0.209	0.246	0.044	0.155	0.101	0.308	0.350	0.497
Hatcher	0.360	0.824	0.838	0.958	0.865	0.749	0.108	0.020	0.223	0.738	0.874	0.258	0.171	0.354	0.001
Yuma	0.784	0.371	0.568	0.329	0.332	0.178	0.593	0.615	0.253	0.017	0.417	0.244	0.366	0.659	0.569
CO08RWA50	0.279	0.302	0.080	0.264	0.152	0.039	0.585	0.478	0.683	0.232	0.275	0.218	0.266	0.323	0.594
CO03765	0.431	0.508	0.426	0.357	0.464	0.089	0.075	0.024	0.340	0.106	0.212	0.193	0.046	0.359	0.185
Otis	0.194	0.151	0.061	0.289	0.393	0.253	0.541	0.373	0.431	0.336	0.320	0.216	0.284	0.115	0.191
Stoneham	0.506	0.238	0.081	0.259	0.044	0.211	0.666	0.460	0.747	0.038	0.021	0.382	0.548	0.159	0.585
Sidney	0.274	0.287	0.160	0.270	0.370	0.125	0.666	0.672	0.582	0.286	0.290	0.195	0.431	0.013	0.490

Correlation output for plant differentials (continued):

	PI625139	PI626580-4	Hatcher	Yuma	CO08RWA50	CO03765	Otis	Stoneham	Sidney
CO03797	0.148	0.535	0.360	0.784	0.279	0.431	0.194	0.506	0.274
CO03804	0.235	0.061	0.824	0.371	0.302	0.508	0.151	0.238	0.287
CO03811	0.048	0.525	0.838	0.568	0.080	0.426	0.061	0.081	0.160
Yumar	0.230	0.148	0.958	0.329	0.264	0.357	0.289	0.259	0.270
CO950043	0.163	0.297	0.865	0.332	0.152	0.464	0.393	0.044	0.370
CO960223	0.183	0.210	0.749	0.178	0.039	0.089	0.253	0.211	0.125
94M370	0.519	0.471	0.108	0.593	0.585	0.075	0.541	0.665	0.666
Karee-Dn8	0.263	0.209	0.020	0.615	0.478	0.024	0.373	0.460	0.672
Betta-Dn9	0.628	0.246	0.223	0.253	0.683	0.340	0.431	0.747	0.582
KS94WGRC29	0.030	0.044	0.738	0.017	0.232	0.106	0.336	0.038	0.286
Stanton	0.008	0.155	0.874	0.417	0.275	0.212	0.320	0.021	0.290
STARS 9302W	0.532	0.101	0.258	0.244	0.218	0.193	0.216	0.382	0.195
KS92WGRC25	0.280	0.308	0.171	0.366	0.267	0.046	0.284	0.548	0.431
CI2401	0.075	0.350	0.354	0.659	0.323	0.359	0.115	0.159	0.013
PI626197	0.461	0.497	0.001	0.569	0.594	0.185	0.191	0.585	0.490
PI625139	1.000	0.033	0.150	0.156	0.448	0.564	0.408	0.640	0.456
PI626580-4	0.033	1.000	0.215	0.318	0.406	0.332	0.046	0.431	0.120
Hatcher	0.150	0.215	1.000	0.438	0.215	0.377	0.155	0.122	0.195
Yuma	0.156	0.318	0.438	1.000	0.143	0.302	0.103	0.140	0.133
CO08RWA50	0.448	0.406	0.215	0.143	1.000	0.011	0.223	0.558	0.649
CO03765	0.564	0.332	0.377	0.302	0.011	1.000	0.399	0.231	0.318
Otis	0.40	0.046	0.155	0.103	0.223	0.399	1.000	0.193	0.520
Stoneham	0.640	0.431	0.122	0.140	0.558	0.231	0.193	1.000	0.434
Sidney	0.456	0.120	0.195	0.133	0.649	0.318	0.520	0.434	1.000

```
#Calculate Means and Standard Deviations


apply(Diff24b,2,mean)

apply(Diff24b,2,sd)
```

Means and Standard Deviations outputs for plant differentials:

	CO03797	CO03804	CO03811	Yumar	CO950043	CO960223	94M370	Karee-Dn8	Betta-Dn9	KS94WGRC29	Stanton	STARS 9302W	KS92WGRC25	CI2401	PI626197	PI625139	PI626580-4	Hatcher	Yuma	CO08RWA50	CO03765	Otis	Stoneham	Sidney
Mean	6.205	6.996	7.521	6.737	6.850	5.273	3.799	6.300	6.034	5.056	7.514	5.249	6.649	4.583	5.149	5.936	4.186	6.892	8.139	3.481	5.643	8.218	5.352	4.414
SD	0.827	0.784	0.615	0.954	1.022	1.572	0.677	1.030	0.651	1.919	0.895	0.737	0.498	0.628	0.472	0.649	0.636	0.911	0.416	0.422	0.684	0.314	0.558	0.726

2011 isolates: leaf chlorosis

#Read in the data (Screenings2011.csv is the table with the mean values of leaf chlorosis of 2011 isolates biotyped with the 8 set of differentials, obtained with the PROC MIXED procedure (SAS))

Screenings2011.csv

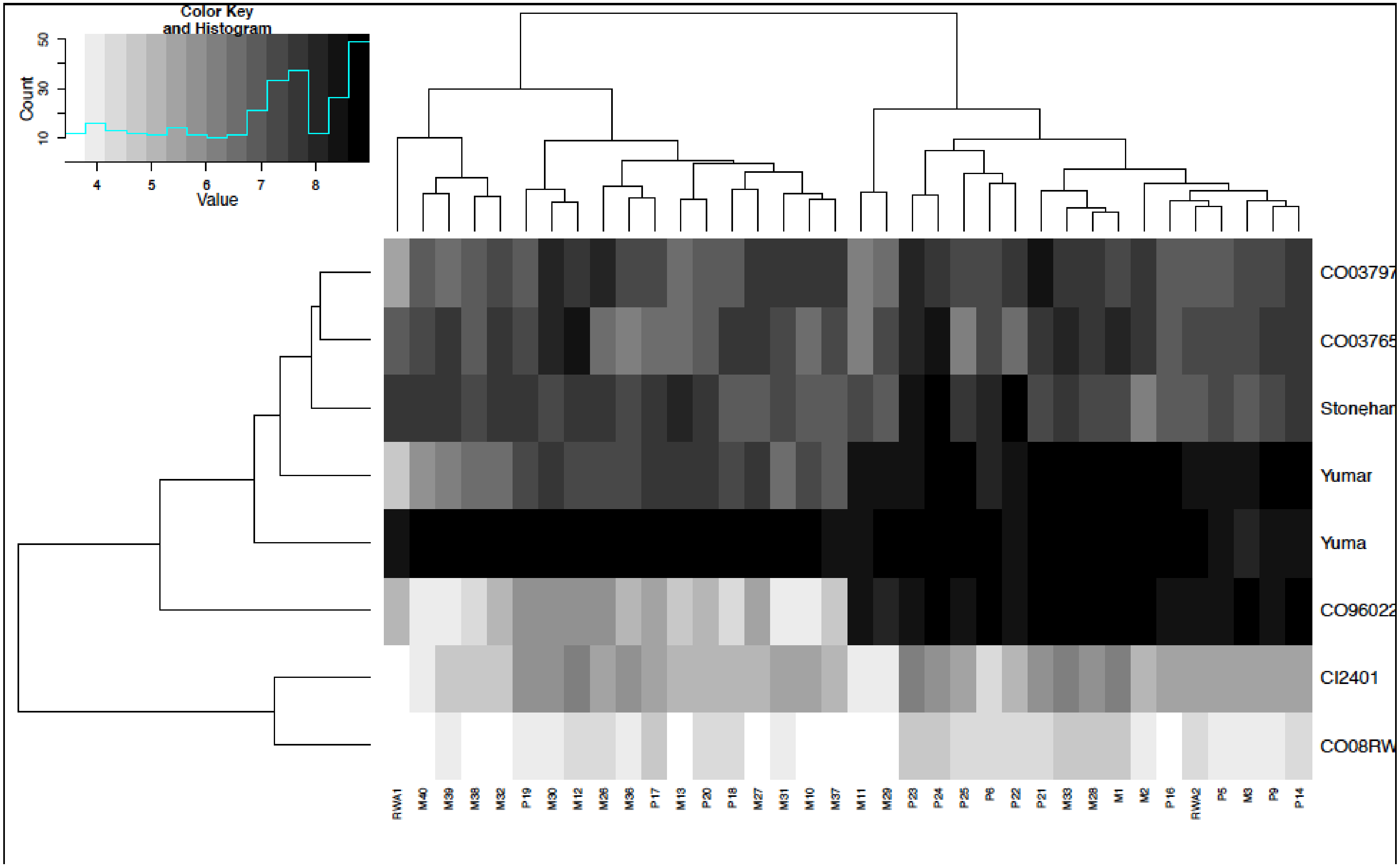
	M 1	M 2	M 3	P 5	P 6	P 9	M 10	M 11	M 12	M 13	P 14	P 16	P 17	P 18	P 19	P 20	P 21	P 22	P 23
CO03797	7.48	7.71	7.39	6.87	7.48	7.35	7.58	6.19	7.70	6.60	7.64	7.10	7.44	6.92	7.06	6.98	8.57	7.58	7.95
Yumar	8.66	8.62	8.38	8.29	8.14	8.63	7.17	8.47	7.14	7.77	8.67	8.82	7.78	7.23	7.46	7.56	8.80	8.44	8.28
CO960223	8.87	8.70	8.80	8.53	8.76	8.57	4.07	8.50	5.97	4.77	8.77	8.51	5.33	4.50	5.68	4.96	8.93	8.37	8.57
CI2401	6.28	5.27	5.48	5.44	4.47	5.63	5.40	4.07	6.02	4.97	5.54	5.40	5.58	5.27	5.74	5.08	5.86	5.01	6.31
Yuma	8.90	8.73	8.18	8.55	8.75	8.49	8.78	8.49	8.77	8.73	8.50	8.87	8.90	8.64	8.90	8.70	8.80	8.59	8.81
CO08RWA50	4.73	3.97	4.08	4.17	4.44	3.82	3.74	3.43	4.40	3.52	4.43	3.77	4.56	4.20	4.00	4.23	4.50	4.30	4.73
CO03765	7.88	7.64	7.18	7.33	7.30	7.69	6.65	6.20	8.34	6.61	7.60	7.12	6.59	7.63	7.44	7.00	7.52	6.61	8.02
Stoneham	7.41	6.37	7.00	7.43	7.90	7.32	7.04	7.20	7.54	7.95	7.58	6.98	7.62	7.04	7.68	7.75	7.24	8.97	8.31

	P 24	P 25	M 26	M 27	M 28	M 29	M 30	M 31	M 32	M 33	M 36	M 37	M 38	M 39	M 40	RWA1	RWA2
CO03797	7.73	7.40	8.18	7.64	7.78	6.67	8.02	7.53	7.43	7.85	7.28	7.73	7.03	6.41	6.83	5.41	7.00
Yumar	8.77	8.96	7.23	7.57	8.62	8.41	7.59	6.39	6.64	8.79	7.26	6.80	6.74	6.10	5.85	4.91	8.52
CO960223	8.60	8.45	5.73	5.32	8.92	8.22	5.77	4.12	5.04	8.63	5.04	4.68	4.23	4.01	3.90	4.93	8.26
CI2401	5.65	5.61	5.47	5.24	5.86	3.87	5.70	5.42	4.69	6.12	5.87	5.14	4.64	4.77	4.11	3.68	5.35
Yuma	8.72	8.86	8.60	8.63	8.78	8.81	8.80	8.76	8.81	8.73	8.89	8.47	8.85	8.63	8.81	8.43	8.70
CO08RWA50	4.83	4.23	4.30	3.70	4.80	3.72	4.13	3.97	3.58	4.85	3.90	3.80	3.70	4.13	3.48	3.70	4.37
CO03765	8.29	6.28	6.60	7.85	7.74	7.13	7.93	7.36	7.50	7.92	6.24	7.17	7.08	7.65	7.22	7.05	7.24
Stoneham	8.95	7.63	7.79	6.93	7.27	6.96	7.25	7.43	7.60	7.79	7.37	6.94	7.24	7.60	7.58	7.63	6.82

```
Screen2011<-read.csv("c:/Documents and Settings/insectaryusr/Desktop/Mariana lab/Hess/Screenings2011.csv",header=T)
#Drop "extra" empty rows
Screen2011<-Screen2011[1:8,]
row.names(Screen2011)<-Screen2011[,1]
Screen2011<-Screen2011[,-1]
Screen2011<-as.matrix(Screen2011)
```

```
#Heatmap

library(gplots)
heatmap.2(Screen2011,col=colorRampPalette(c("white","black"))(15),trace="none")
```



```
#Correlation

cor(Screen2011)
```

Correlation output for 2011 leaf chlorosis:

```
M1       M2       M3       P5       P6       P9       M10
M1   1.0000000 0.9775227 0.9856943 0.9893308 0.9298485 0.9928267 0.5458314
M2   0.9775227 1.0000000 0.9777490 0.9521281 0.9163424 0.9716702 0.5465113
M3   0.9856943 0.9777490 1.0000000 0.9798446 0.9451813 0.9872738 0.4878702
P5   0.9893308 0.9521281 0.9798446 1.0000000 0.9619422 0.9875955 0.5431018
P6   0.9298485 0.9163424 0.9451813 0.9619422 1.0000000 0.9379705 0.5405410
P9   0.9928267 0.9716702 0.9872738 0.9875955 0.9379705 1.0000000 0.5763620
M10  0.5458314 0.5465113 0.4878702 0.5431018 0.5405410 0.5763620 1.0000000
M11  0.9509109 0.9166921 0.9560522 0.9792053 0.9667562 0.9477257 0.4988356
M12  0.6950575 0.6811112 0.6278688 0.6855811 0.6706325 0.7215597 0.9105428
M13  0.6765546 0.6282702 0.6212869 0.7081473 0.6965201 0.7089221 0.9361490
P14  0.9823982 0.9702794 0.9925739 0.9879214 0.9686715 0.9918997 0.5499966
P16  0.9914104 0.9738344 0.9827219 0.9866624 0.9353098 0.9866033 0.5814631
P17  0.6502598 0.6334158 0.6038554 0.6689099 0.6746560 0.6762813 0.9706937
P18  0.5867838 0.5777212 0.5071139 0.5876165 0.5756784 0.6139027 0.9610259
P19  0.7407019 0.6989329 0.6731902 0.7504783 0.7219200 0.7629192 0.9396228
P20  0.6449258 0.6160854 0.5880640 0.6727421 0.6822892 0.6776241 0.9570718
```

```
P21  0.9577248 0.9800979 0.9798685 0.9413857 0.9280654 0.9646650 0.5783929
P22  0.8635783 0.8141264 0.8866418 0.9173668 0.9499799 0.8894566 0.5968288
P23  0.9569117 0.9181698 0.9498837 0.9679681 0.9469243 0.9729149 0.6465720
P24  0.9130460 0.8673005 0.9131950 0.9510644 0.9584452 0.9425537 0.6097753
P25  0.9347214 0.9069291 0.9460827 0.9503562 0.9218946 0.9362123 0.5861057
M26  0.6624815 0.6601814 0.6449962 0.6792613 0.7197046 0.6990132 0.9447339
M27  0.7298835 0.7402203 0.6766055 0.7163626 0.7027184 0.7598920 0.9407307
M28  0.9899926 0.9909957 0.9937225 0.9813062 0.9537409 0.9868478 0.5348334
M29  0.9570435 0.9491395 0.9518179 0.9749981 0.9787904 0.9562262 0.5729815
M30  0.7282495 0.7381867 0.6790833 0.7142219 0.7058701 0.7580607 0.9455741
M31  0.4810329 0.4707903 0.4114879 0.4851552 0.5018507 0.5134024 0.9701006
M32  0.6668964 0.6531484 0.6143205 0.6822641 0.7117551 0.6978993 0.9379601
M33  0.9863521 0.9704604 0.9854097 0.9871135 0.9550465 0.9970760 0.6078358
M36  0.6379820 0.6077841 0.5820014 0.6437554 0.6213974 0.6572021 0.9723940
M37  0.6119807 0.6243751 0.5615267 0.6060567 0.6218011 0.6450412 0.9789252
M38  0.5943255 0.5827615 0.5287182 0.6085886 0.6285349 0.6217578 0.9724587
M39  0.4690516 0.4328261 0.3822042 0.4957943 0.5200809 0.4964895 0.9000113
M40  0.5313490 0.5095202 0.4649336 0.5584660 0.6046948 0.5605968 0.9272867
RWA1 0.5670431 0.5027445 0.4932913 0.6196957 0.6743669 0.5766397 0.7395017
RWA2 0.9874260 0.9793225 0.9750304 0.9830724 0.9426894 0.9790521 0.5764958
           M11       M12       M13       P14       P16       P17       P18
M1   0.9509109 0.6950575 0.6765546 0.9823982 0.9914104 0.6502598 0.5867838
```

```
M2   0.9166921 0.6811112 0.6282702 0.9702794 0.9738344 0.6334158 0.5777212
M3   0.9560522 0.6278688 0.6212869 0.9925739 0.9827219 0.6038554 0.5071139
P5   0.9792053 0.6855811 0.7081473 0.9879214 0.9866624 0.6689099 0.5876165
P6   0.9667562 0.6706325 0.6965201 0.9686715 0.9353098 0.6746560 0.5756784
P9   0.9477257 0.7215597 0.7089221 0.9918997 0.9866033 0.6762813 0.6139027
M10  0.4988356 0.9105428 0.9361490 0.5499966 0.5814631 0.9706937 0.9610259
M11  1.0000000 0.5894682 0.6899963 0.9674886 0.9692749 0.6584305 0.5278764
M12  0.5894682 1.0000000 0.8878189 0.6842811 0.6810084 0.8772382 0.9523145
M13  0.6899963 0.8878189 1.0000000 0.6913034 0.7141397 0.9787501 0.9370566
P14  0.9674886 0.6842811 0.6913034 1.0000000 0.9832238 0.6660371 0.5792653
P16  0.9692749 0.6810084 0.7141397 0.9832238 1.0000000 0.6979919 0.6037299
P17  0.6584305 0.8772382 0.9787501 0.6660371 0.6979919 1.0000000 0.9336618
P18  0.5278764 0.9523145 0.9370566 0.5792653 0.6037299 0.9336618 1.0000000
P19  0.6931950 0.9619431 0.9727980 0.7316806 0.7525114 0.9553469 0.9584548
P20  0.6495466 0.9154741 0.9909266 0.6633549 0.6794645 0.9816842 0.9665627
P21  0.9155124 0.6773555 0.6511453 0.9741885 0.9633978 0.6685526 0.5643087
P22  0.9395121 0.6512509 0.7755940 0.9202497 0.8884554 0.7425170 0.5825610
P23  0.9203627 0.7951971 0.7711427 0.9688094 0.9455214 0.7358237 0.6700517
P24  0.9296519 0.7550362 0.7861522 0.9524312 0.9112013 0.7234193 0.6650706
P25  0.9699522 0.6063174 0.7326042 0.9497808 0.9675965 0.7291972 0.5550082
M26  0.6528036 0.8917868 0.9262568 0.7008072 0.6957223 0.9613235 0.8808474
M27  0.6450964 0.9755484 0.9169025 0.7308562 0.7382602 0.9233429 0.9652298
```

M28	0.9550332	0.6747428	0.6517207	0.9912753	0.9869272	0.6421798	0.5649704
M29	0.9769748	0.6896798	0.7246233	0.9729424	0.9673472	0.7020237	0.6291993
M30	0.6402357	0.9796684	0.9119108	0.7307205	0.7348508	0.9252841	0.9556144
M31	0.4168249	0.9440585	0.8995399	0.4840370	0.4932688	0.9169026	0.9642847
M32	0.6237005	0.9756024	0.9331096	0.6834191	0.6732154	0.9328523	0.9569763
M33	0.9558789	0.7364291	0.7367632	0.9959263	0.9864077	0.7105316	0.6379424
M36	0.6115539	0.8836341	0.9562337	0.6324299	0.6757623	0.9826890	0.9149734
M37	0.5426016	0.9633127	0.9153493	0.6230291	0.6283861	0.9441010	0.9658934
M38	0.5637255	0.9503358	0.9524196	0.6027064	0.6162547	0.9611963	0.9845104
M39	0.4364343	0.9233603	0.8932506	0.4697027	0.4726572	0.8705416	0.9576926
M40	0.5081139	0.9404352	0.9121744	0.5471237	0.5406479	0.9100385	0.9545320
RWA1	0.5775055	0.8512319	0.8144981	0.5725684	0.5526371	0.7701777	0.8224278
RWA2	0.9687351	0.6850256	0.7067204	0.9790188	0.9952043	0.6926742	0.6157937

	P19	P20	P21	P22	P23	P24	P25
M1	0.7407019	0.6449258	0.9577248	0.8635783	0.9569117	0.9130460	0.9347214
M2	0.6989329	0.6160854	0.9800979	0.8141264	0.9181698	0.8673005	0.9069291
M3	0.6731902	0.5880640	0.9798685	0.8866418	0.9498837	0.9131950	0.9460827
P5	0.7504783	0.6727421	0.9413857	0.9173668	0.9679681	0.9510644	0.9503562
P6	0.7219200	0.6822892	0.9280654	0.9499799	0.9469243	0.9584452	0.9218946
P9	0.7629192	0.6776241	0.9646650	0.8894566	0.9729149	0.9425537	0.9362123
M10	0.9396228	0.9570718	0.5783929	0.5968288	0.6465720	0.6097753	0.5861057
M11	0.6931950	0.6495466	0.9155124	0.9395121	0.9203627	0.9296519	0.9699522

M12	0.9619431	0.9154741	0.6773555	0.6512509	0.7951971	0.7550362	0.6063174
M13	0.9727980	0.9909266	0.6511453	0.7755940	0.7711427	0.7861522	0.7326042
P14	0.7316806	0.6633549	0.9741885	0.9202497	0.9688094	0.9524312	0.9497808
P16	0.7525114	0.6794645	0.9633978	0.8884554	0.9455214	0.9112013	0.9675965
P17	0.9553469	0.9816842	0.6685526	0.7425170	0.7358237	0.7234193	0.7291972
P18	0.9584548	0.9665627	0.5643087	0.5825610	0.6700517	0.6650706	0.5550082
P19	1.0000000	0.9740893	0.7052358	0.7538149	0.8272875	0.8040802	0.7261880
P20	0.9740893	1.0000000	0.6334955	0.7363253	0.7453654	0.7610615	0.6836849
P21	0.7052358	0.6334955	1.0000000	0.8694587	0.9364483	0.8810230	0.9356914
P22	0.7538149	0.7363253	0.8694587	1.0000000	0.9305153	0.9537285	0.9380988
P23	0.8272875	0.7453654	0.9364483	0.9305153	1.0000000	0.9691057	0.9133940
P24	0.8040802	0.7610615	0.8810230	0.9537285	0.9691057	1.0000000	0.8886381
P25	0.7261880	0.6836849	0.9356914	0.9380988	0.9133940	0.8886381	1.0000000
M26	0.9286918	0.9371669	0.7288883	0.7840153	0.7894202	0.7554420	0.7313954
M27	0.9629848	0.9415910	0.7393045	0.6848875	0.7992882	0.7697176	0.6793666
M28	0.7107474	0.6301838	0.9816527	0.8752563	0.9503931	0.9125644	0.9379899
M29	0.7502386	0.7102001	0.9302713	0.9075610	0.9302041	0.9405336	0.9310227
M30	0.9634603	0.9365671	0.7479840	0.6941940	0.8086898	0.7674621	0.6832145
M31	0.9300211	0.9362032	0.4945999	0.5403611	0.6193386	0.5886881	0.4715556
M32	0.9697694	0.9616689	0.6720785	0.7210971	0.7901564	0.7760575	0.6427618
M33	0.7813042	0.7096930	0.9714380	0.9126136	0.9777430	0.9545873	0.9468350
M36	0.9551155	0.9519796	0.6492436	0.7023817	0.7285450	0.6777384	0.7059047

```
M37  0.9504859 0.9487515 0.6500660 0.6361258 0.7212545 0.6831288 0.6014887
M38  0.9666854 0.9817994 0.5941959 0.6523896 0.7036033 0.6957261 0.5972366
M39  0.9163922 0.9311936 0.4246578 0.5397505 0.6042200 0.6201714 0.4312145
M40  0.9356971 0.9504837 0.5237957 0.6256626 0.6736537 0.6765452 0.5179156
RWA1 0.8541646 0.8430654 0.4916340 0.6655541 0.7010739 0.7270792 0.5152329
RWA2 0.7480322 0.6816499 0.9562074 0.8723313 0.9326206 0.9069586 0.9506575
           M26       M27       M28       M29       M30       M31       M32
M1   0.6624815 0.7298835 0.9899926 0.9570435 0.7282495 0.4810329 0.6668964
M2   0.6601814 0.7402203 0.9909957 0.9491395 0.7381867 0.4707903 0.6531484
M3   0.6449962 0.6766055 0.9937225 0.9518179 0.6790833 0.4114879 0.6143205
P5   0.6792613 0.7163626 0.9813062 0.9749981 0.7142219 0.4851552 0.6822641
P6   0.7197046 0.7027184 0.9537409 0.9787904 0.7058701 0.5018507 0.7117551
P9   0.6990132 0.7598920 0.9868478 0.9562262 0.7580607 0.5134024 0.6978993
M10  0.9447339 0.9407307 0.5348334 0.5729815 0.9455741 0.9701006 0.9379601
M11  0.6528036 0.6450964 0.9550332 0.9769748 0.6402357 0.4168249 0.6237005
M12  0.8917868 0.9755484 0.6747428 0.6896798 0.9796684 0.9440585 0.9756024
M13  0.9262568 0.9169025 0.6517207 0.7246233 0.9119108 0.8995399 0.9331096
P14  0.7008072 0.7308562 0.9912753 0.9729424 0.7307205 0.4840370 0.6834191
P16  0.6957223 0.7382602 0.9869272 0.9673472 0.7348508 0.4932688 0.6732154
P17  0.9613235 0.9233429 0.6421798 0.7020237 0.9252841 0.9169026 0.9328523
P18  0.8808474 0.9652298 0.5649704 0.6291993 0.9556144 0.9642847 0.9569763
P19  0.9286918 0.9629848 0.7107474 0.7502386 0.9634603 0.9300211 0.9697694
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P20  0.9371669 0.9415910 0.6301838 0.7102001 0.9365671 0.9362032 0.9616689
P21  0.7288883 0.7393045 0.9816527 0.9302713 0.7479840 0.4945999 0.6720785
P22  0.7840153 0.6848875 0.8752563 0.9075610 0.6941940 0.5403611 0.7210971
P23  0.7894202 0.7992882 0.9503931 0.9302041 0.8086898 0.6193386 0.7901564
P24  0.7554420 0.7697176 0.9125644 0.9405336 0.7674621 0.5886881 0.7760575
P25  0.7313954 0.6793666 0.9379899 0.9310227 0.6832145 0.4715556 0.6427618
M26  1.0000000 0.9168814 0.6740258 0.6995214 0.9353351 0.9142632 0.9480626
M27  0.9168814 1.0000000 0.7225829 0.7420327 0.9970759 0.9268734 0.9640663
M28  0.6740258 0.7225829 1.0000000 0.9698293 0.7230744 0.4660341 0.6619950
M29  0.6995214 0.7420327 0.9698293 1.0000000 0.7333473 0.5137777 0.7121249
M30  0.9353351 0.9970759 0.7230744 0.7333473 1.0000000 0.9353057 0.9707219
M31  0.9142632 0.9268734 0.4660341 0.5137777 0.9353057 1.0000000 0.9624840
M32  0.9480626 0.9640663 0.6619950 0.7121249 0.9707219 0.9624840 1.0000000
M33  0.7366234 0.7797469 0.9875031 0.9675638 0.7789009 0.5439113 0.7273245
M36  0.9563720 0.9084665 0.6156994 0.6439338 0.9192315 0.9244720 0.9193399
M37  0.9545982 0.9760749 0.6113205 0.6388111 0.9834466 0.9773756 0.9772470
M38  0.9360398 0.9563912 0.5838673 0.6529235 0.9567838 0.9794080 0.9831772
M39  0.8356915 0.8856108 0.4435735 0.5388901 0.8822228 0.9619963 0.9480700
M40  0.9067362 0.9134330 0.5224378 0.6044773 0.9183979 0.9753441 0.9805009
RWA1 0.7695773 0.7768635 0.5470668 0.6531991 0.7813935 0.8316354 0.8959867
RWA2 0.6830364 0.7440488 0.9878898 0.9815532 0.7369170 0.4950718 0.6793486
           M33       M36       M37       M38       M39       M40      RWA1
```

M1	0.9863521	0.6379820	0.6119807	0.5943255	0.4690516	0.5313490	0.5670431
M2	0.9704604	0.6077841	0.6243751	0.5827615	0.4328261	0.5095202	0.5027445
M3	0.9854097	0.5820014	0.5615267	0.5287182	0.3822042	0.4649336	0.4932913
P5	0.9871135	0.6437554	0.6060567	0.6085886	0.4957943	0.5584660	0.6196957
P6	0.9550465	0.6213974	0.6218011	0.6285349	0.5200809	0.6046948	0.6743669
P9	0.9970760	0.6572021	0.6450412	0.6217578	0.4964895	0.5605968	0.5766397
M10	0.6078358	0.9723940	0.9789252	0.9724587	0.9000113	0.9272867	0.7395017
M11	0.9558789	0.6115539	0.5426016	0.5637255	0.4364343	0.5081139	0.5775055
M12	0.7364291	0.8836341	0.9633127	0.9503358	0.9233603	0.9404352	0.8512319
M13	0.7367632	0.9562337	0.9153493	0.9524196	0.8932506	0.9121744	0.8144981
P14	0.9959263	0.6324299	0.6230291	0.6027064	0.4697027	0.5471237	0.5725684
P16	0.9864077	0.6757623	0.6283861	0.6162547	0.4726572	0.5406479	0.5526371
P17	0.7105316	0.9826890	0.9441010	0.9611963	0.8705416	0.9100385	0.7701777
P18	0.6379424	0.9149734	0.9658934	0.9845104	0.9576926	0.9545320	0.8224278
P19	0.7813042	0.9551155	0.9504859	0.9666854	0.9163922	0.9356971	0.8541646
P20	0.7096930	0.9519796	0.9487515	0.9817994	0.9311936	0.9504837	0.8430654
P21	0.9714380	0.6492436	0.6500660	0.5941959	0.4246578	0.5237957	0.4916340
P22	0.9126136	0.7023817	0.6361258	0.6523896	0.5397505	0.6256626	0.6655541
P23	0.9777430	0.7285450	0.7212545	0.7036033	0.6042200	0.6736537	0.7010739
P24	0.9545873	0.6777384	0.6831288	0.6957261	0.6201714	0.6765452	0.7270792
P25	0.9468350	0.7059047	0.6014887	0.5972366	0.4312145	0.5179156	0.5152329
M26	0.7366234	0.9563720	0.9545982	0.9360398	0.8356915	0.9067362	0.7695773

```
M27  0.7797469 0.9084665 0.9760749 0.9563912 0.8856108 0.9134330 0.7768635
M28  0.9875031 0.6156994 0.6113205 0.5838673 0.4435735 0.5224378 0.5470668
M29  0.9675638 0.6439338 0.6388111 0.6529235 0.5388901 0.6044773 0.6531991
M30  0.7789009 0.9192315 0.9834466 0.9567838 0.8822228 0.9183979 0.7813935
M31  0.5439113 0.9244720 0.9773756 0.9794080 0.9619963 0.9753441 0.8316354
M32  0.7273245 0.9193399 0.9772470 0.9831772 0.9480700 0.9805009 0.8959867
M33  1.0000000 0.6840832 0.6749211 0.6539225 0.5250807 0.5950575 0.6028068
M36  0.6840832 1.0000000 0.9402796 0.9439231 0.8546322 0.8935575 0.7493898
M37  0.6749211 0.9402796 1.0000000 0.9802349 0.9170442 0.9528505 0.7938979
M38  0.6539225 0.9439231 0.9802349 1.0000000 0.9646153 0.9831659 0.8667885
M39  0.5250807 0.8546322 0.9170442 0.9646153 1.0000000 0.9854980 0.9210943
M40  0.5950575 0.8935575 0.9528505 0.9831659 0.9854980 1.0000000 0.9251751
RWA1 0.6028068 0.7493898 0.7938979 0.8667885 0.9210943 0.9251751 1.0000000
RWA2 0.9805629 0.6590887 0.6304519 0.6251060 0.4889032 0.5533211 0.5742080
          RWA2
M1   0.9874260
M2   0.9793225
M3   0.9750304
P5   0.9830724
P6   0.9426894
P9   0.9790521
M10  0.5764958
```

M11	0.9687351
M12	0.6850256
M13	0.7067204
P14	0.9790188
P16	0.9952043
P17	0.6926742
P18	0.6157937
P19	0.7480322
P20	0.6816499
P21	0.9562074
P22	0.8723313
P23	0.9326206
P24	0.9069586
P25	0.9506575
M26	0.6830364
M27	0.7440488
M28	0.9878898
M29	0.9815532
M30	0.7369170
M31	0.4950718
M32	0.6793486
M33	0.9805629

```
M36  0.6590887
M37  0.6304519
M38  0.6251060
M39  0.4889032
M40  0.5533211
RWA1 0.5742080
RWA2 1.0000000
```

```
#Calculate Means and Standard Deviations

apply(Screen2011,2,mean)
apply(Screen2011,2,sd)
```

Means and Standard Deviations outputs for 2011 leaf chlorosis:

	M 1	M 2	M 3	P 5	P 6	P 9	M 10	M 11	M 12	M 13	P 14	P 16	P 17	P 18	P 19	P 20	P 21	P 22	P 23
Mean	7.526	7.123	7.062	7.075	7.154	7.185	6.303	6.569	6.984	6.364	7.340	7.070	6.724	6.427	6.744	6.532	7.525	7.233	7.622
SD	1.438	1.768	1.581	1.565	1.746	1.681	1.754	1.992	1.439	1.806	1.560	1.776	1.470	1.585	1.526	1.582	1.616	1.758	1.391

	P 24	P 25	M 26	M 27	M 28	M 29	M 30	M 31	M 32	M 33	M 36	M 37	M 38	M 39	M 40	RWA1	RWA2
Mean	7.690	7.177	6.737	6.609	7.471	6.723	6.899	6.371	6.413	7.584	6.480	6.340	6.190	6.163	5.973	5.718	7.031
SD	1.574	1.682	1.488	1.679	1.467	1.959	1.553	1.728	1.784	1.400	1.559	1.619	1.789	1.738	1.960	1.790	1.536