Appendix 2. (Continued) Alignment of our ITS sequence variants to Genbank accessions (FJ426346-FJ426357) identified in Sturtevant et al. 2009. "?'s" represent portions of the ITS gene not analyzed in our study.

	751
FJ426346_SpiMI1	G A G G A
FJ426347_SpiMI2	G A G G A
FJ426348_SpiMI3	G A G G A
FJ426349_SpiMI4	G A G G A
FJ426350_SpiMI6	G A G G A
FJ426351_SpiMI7	G A G G A
EWM1	? ? ? ? ?
EWM2	? ? ? ? ?
EWM3	? ? ? ? ?
FJ426352_SibMI1	G A G G A
FJ426353_SibMI2	G A G G A
FJ426354_SibMI3	G A G G A
FJ426355_SibMI4	G A G G A
FJ426356_SibMI5	G A G G A
FJ426357_SibMI6	G A G G A
NWM1	? ? ? ? ?
NWM2	**? ? ? ? ?**
NWM3	**? ? ? ? ?**

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Invasions and Impacts of Alligatorweed in the Upper Xiaoqing River Basin of Northern China

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ABSTRACT

Alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb), is a problematic and difficult to manage invasive weed. The recent invasion in the upper Xiaoqing River, northern China extends its range northwards through almost five degrees latitude and 500 km from the northern limit and main invasion area of the weed in China. The length of main branches of the weed in Jinan ranges from 198 cm to 382 cm, with an average value of 266.67 ± 24.01 cm. The average number of nodes and adventitious roots on the main branches are 27.01 ± 2.25 and 17.11 ± 0.84, respectively. The number of main branches per linear meter transect is 376-511, with an average of 436.52 ± 55.33. The main impact of alligatorweed is that it chokes the flood flow of the local river in rainy seasons, but was not found to cause obvious damage to agricultural production in the area covered by this study. However, the presence of this weed in northern China highlights its potential future risk, and questions the previous models used to predict the spread and distribution of this weed.

Key Words: *Alternanthera philoxeroides*, extend, northern limit, five degrees latitude, 500 km.

INTRODUCTION

Alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb), is a very difficult to manage invasive weed. It originated in the Parana River region of South America (Maddox 1968, Vogt et al. 1979) and was spread to the other areas of South America, North America, Asia, Australia and some adjacent island countries (Julien et al. 1995). It grows in both aquatic and terrestrial habitats, and in some areas it blankets water surfaces,

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and chokes waterways and ponds, and can also invade farm land. Alligatorweed was introduced to China's mainland in the late 1930s as horse feed by Japanese in Shanghai suburbs (Diao 1990), and was cultivated widely as a forage in southern China in the 1950s. Unfortunately it escaped cultivation and now is a significant ecological problem. It is currently listed on a shortlist of 16 invasive species requiring special control in China (State Environmental Protection Administration of China 2003).

Julien et al. (1995) used a climate matching program, CLIMEX, and the known distribution of alligatorweed in South and North America to infer areas suitable for its growth in the world, and the results showed that the northern limit in the region of China for the weed is about 32 degrees north latitude (Figure 1). Its main distribution in China is the southern areas along the middle and lower reaches of the Yangtze River (Ma and Wang 2005). The Yangtze River is the longest river in China, and the latitude of the main cities along its middle and lower reaches is from about 28 to 32 degrees north. Latitude and longitude of Jinan and main cities along the middle-lower reaches of the Yangtze River and main meteorological parameters are shown in Table 1 (data from China Meteorological Data Sharing Service System, cdc. cma. gov. cn). In contrast to predictions by Julien et al. (1995) and earlier documents in China (Ma and Wang 2005), we found alligatorweed to have significantly invaded the upper Xiaqing River in Jinan of Shandong province, located at 36.7 degrees north latitude (Liu et al. 2006). These later surveys suggest that the main invasion area of alligatorweed in China had moved north by almost 5 degrees latitude and nearly 500 km indicting a greater range than was previously predicted.

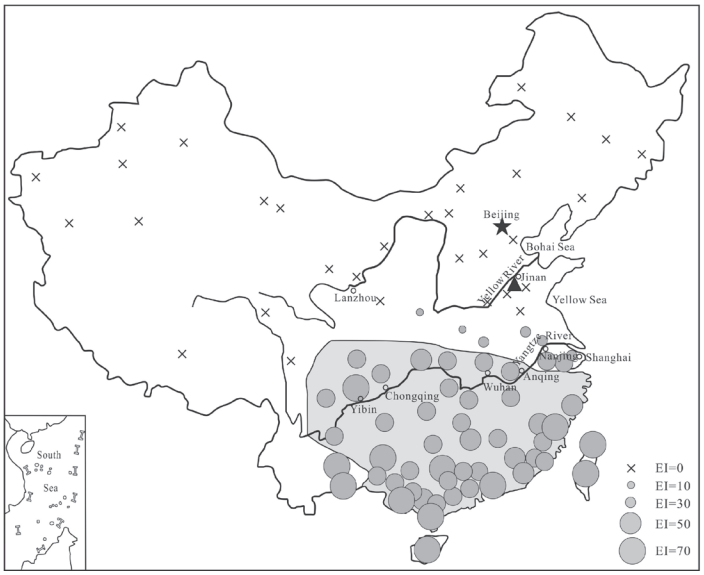

Figure 1. Location map showing the predicted and known historical distribution of alligatorweed in China (Julien et al. 1995) and our present field survey in Jinan, northern China (predicted distribution indicated by circle and cross, and known historical distribution by shadow, our present work by triangle). The map is based on the result and figure of Julien et al. (1995) with some modification. The annual ecoclimatic index (EI) describes the potential growth of the weed and is scaled between 0 and 100. The values of EI above 10 for locations favorable to the growth of alligatorweed are indicated by circles, and the areas of circles are proportional to the predicted suitability of the location. The values of EI between 0 and 10 for locations unfavorable for the weed are indicated by crosses. For a full description refer to Julien et al. (1995).

The primary invasion sites of alligatorweed were found near the source of the Xiaoqing River, however none of the general stand characteristics, for example stand density or distribution to the other parts of the upper Xiaoqing river, were known. The impact of these introductions on local rivers and agricultural production were also unknown. To answer these questions, we conducted a further field investigation during 2006 to 2007. In addition, the upper Xiaoqing River is very different in water quality depending on the distance from the source, with clear unpolluted waters in the region near the source to polluted waters through most parts of the upper Xiaoqing River. Thus, we also wanted to test the hypothesis that the distribution of alligatorweed is not limited by water quality in the upper Xiaoqing River.

MATERIALS AND METHODS

Study Sites and Scope of Investigation

Jinan, the provincial capital of Shandong, is located in North China on the south bank of the lower reaches of the Yellow River, the second longest river in China. The average annual temperature in Jinan is 14.7 C with an average of -0.4 C in January and 27.5 C in July. The average annual rainfall is 672.7 mm.

The Xiaoqing River is an important river in Shandong province, with a total length of 237 km, of which 70.3 km traverses in Jinan. The Xiaoqing River serves regionally for flood discharge, irrigation, and sewage discharge etc. The Xiaoqing River stems from Muli gate, which is located in the western suburbs of Jinan, then flows eastward through Jinan, Binzhou, Zibo, Dongying and Weifang, and finally joins into the Bohai Sea in Yangjiaokou, Weifang (Figure 2).

The 40 km-long Yufu River is a small seasonal river that mainly discharges water from hills of southern Jinan and then flows northward through Jinan suburban Zhonggong, Dangjia, Duandian, Pingandian, Wujiapu and finally joins into the Yellow River in Beidianzi. It can also flow into the Xiaoqing River through Muli gate.

Field Survey

The investigation was carried out over the Jinan section of the Xiaoqing River, from the source at the Muli gate to the end at Dashaliu gate (70 km), and over 10 km of the Yufu River. The field work was conducted in 2006 and 2007. We recorded the distribution and invasion of alligatorweed at the upper reaches of the Xiaoqing River and the Yufu River. We also interviewed local river management officers, local farmers, and local aquaculture peasants to investigate the impacts of alligatorweed on local rivers and agricultural production. Additionally, we randomly selected five quadrants of rice field (100 m^2), five quadrants of vegetable production areas (≥50 m^2) and five fishponds (≥200 m^2) to survey for the presence of the weed within the cultivated areas. The rice fields were located outside of the river bank and the vegetable production areas were located inside of the bank. The position of

Table 1. Latitude and longitude of Jinan and main cities along the middle-lower reaches of the Yangtze River and their main meteorological parameters

Cities/Parameters	Jinan	Shanghai	Nanjing	Anqing	Wuhan	Chongqing	Yibing
Latitude (N)	36.6	31.4	32.0	30.5	30.6	29.6	28.8
Longitude (E)	117.1	121.5	118.8	117.1	114.1	106.5	104.6
Lowest temperature (°C)	-14.9	-7.7	-13.1	-9.0	-18.1	-1.7	-1.7
Highest temperature (°C)	40.5	37.8	39.7	39.5	39.3	41.9	39.5
Mean temperature (°C)	14.7	16.6	15.4	16.7	16.6	18.2	17.8
Mean temperature of January (°C)	-0.4	4.7	2.4	4.0	3.7	7.8	7.8
annual precipitation (mm)	672.7	1184.4	1062.4	1474.9	1269.0	1104.5	1063.1

survey points was determined by GPS, and digital photos were taken. For the location of field survey points see Figure 2.

Data Collection and Measurements

At the field sites near Peng Zhuang in the upper Xiaoqing River, alligatorweed was the dominant community. We randomly selected three quadrants in the filed sites, and chose six or seven main stem branches that were as long and complete as possible in each quadrant for our data collection. The length, number of branches, number of nodes, number of adventitious roots, and the number of flowers on representative main branches were measured. Small branches with lengths below 5 cm were treated as one class and not measured individually. Because of the creeping, long and interlaced branching of alligatorweed which made counting difficult and counting confusion, we used the transects instead of 1 m^2 quadrants in the present study. We selected six 1m wide transects at random, cut all of the weeds within each transect, and counted the number of main branches in each unit.

Water Quality Data Collection

The recorded water quality data from 2005 to 2007 for the following four monitoring sites in the upper Xiaoqing River were used: Muli gate, Huanxiangdian, Damatou and Xinfeng Zhuang (Jinan Environmental Protection Bureau 2006, 2007, 2008). Muli gate is located at the source of the Xiaoqing River, while Xinfeng Zhuang is close to the Jinan eastern boundary. For the four water quality monitoring sites see Figure 2.

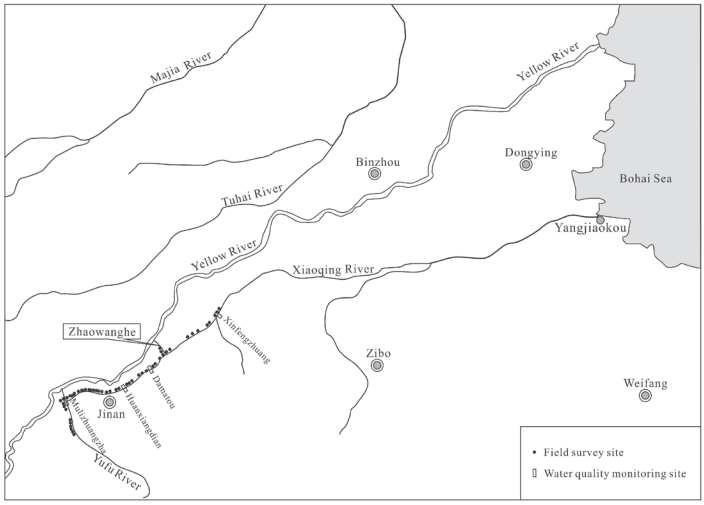

Figure 2. Location map of the Xiaoqing River, field survey points and water quality monitoring sites.

RESULTS AND DISCUSSION

Local Invasions

Alligatorweed was found along the entire study area (Figure 2). In the sections with greater infestation, the weed extended 5-6 m from both sides of the river bank, and occasionally covered the whole river surface where the river was narrow. It formed the dominant community at Peng Zhuang, West Second Circle Road Bridge, Ban Bridge and Huangtai. The infestation in the Yufu River and the Zhaowanghe was particularly serious. The Yufu River is the upstream branch of the Xiaoqing River in western Jinan, and the latter is a small waterway for flood discharge and irrigation between the Xiaoqing River and the Yellow River in eastern Jinan. The 3 km-long part of the Yufu River and a 1 km-long portion of the Zhaowanghe were covered or almost covered by mats of alligatorweed and the flow speed was slow or non-existent in these areas.

In the sections with low infestation, such as Shahe Bridge, Xiaoxu Jia, Hanguan Zhuang Bridge, Zong Jia Bridge and Fu Jia Bridge, the weed was only distributed in small patches. Between the two extremes mentioned above, a sheet-like distribution generally extended 3-5m from the river bank, and sometimes continued 100-200 m along the river.

Compared with the previously predicted northern distribution limits (Julien et al. 1995) and known main invasion areas in southern China (Ma and Wang 2005), the distribution of alligatorweed in the upper Xiaoqing River in Jinan has moved north almost five degrees latitude and nearly 500 km. The average temperature in the coldest month (January) is -0.4 C in Jinan, and 2.4-7.8 C in Shanghai, Nanjing, Anqing, Wuhan, Chongqing, and Yibing along the middle-lower reaches of the Yangzi River (data from China Meteorological Data Sharing Service System , cdc. cma. gov. cn).

Our survey of the upper Xiaoqing River showed that the natural condition of northern China were suitable for the alligatorweed, in contrast to the previous CLIMEX prediction (Julien et al. 1995). CLIMEX is a modeling package employed to predict the potential species distribution based on its current distribution and a huge database of meteorological information (Sutherst et al. 1999, Kriticos et al. 2003, Peterson 2003), and it features a climate-matching function with species-specific responses to key environmental param-

eters (Pattison and Mack 2008). However, CLIMEX has its limitations. As one of climate models, CLIMEX greatly relies on the number and distribution of meteorological stations (Poutsma et al. 2008). Areas with a small number of stations may not give a representative view of the climate in that region because the location of the meteorological stations is frequently unrepresentative for the surrounding area (Bennett et al. 1998).

The reason for the failed distribution predictions of alligatorweed may have been the lack of available meteorological data from northern China. Recently, interest in species distribution models of plant and animals has grown dramatically (Guisan and Thuiller 2005), and the current work of this kind of model could use climatic data of temperature and rainfall from 45000 locations and nearly 25000 locations separately, from www.worldclim.org. The predictions by Julien et al. (1995) used meteorological data from only 2500 locations. In addition, McFadyen (1991) pointed out that predictions of the likely exotic distribution of an organism based solely on knowledge of its native range may be quite erroneous. Julien et al. (1995) just used the distribution data of alligatorweed from South and North America to infer areas suitable for the growth of this weed in the whole world, but they did not use data from Asian, African and European infestations to build his model. This may be another reason that led to the inaccurate predictions in China.

Water Quality in Investigated Section

The distribution of alligatorweed was found in different water qualities from un-polluted to polluted (Figure 3 and 4). A large amount of wastewater is poured into the Xiaoqing River when it flows through Jinan. Although the majority of the wastewater is treated in wastewater plants, excluding the region near the source, most of the surveyed regions of the upper Xiaoqing River were still polluted. According to the 2005-2007 Jinan Environmental Status Bulletin (Jinan Environmental Protection Bureau, 2006, 2007, 2008), the average values of chemical oxygen demand (CODcr), biochemical oxygen demand (BOD_5), ammonia nitrogen, volatile phenols, cyanide, arsenic, Hg, Cr^{6+} and Pb in Muli gate comply with the limit value of Grade III of the "Environmental Quality Standard for Surface water (GB3838-2002) "(State Environmental Protection Administration of China 2002), and shows that the water quality at the source of the Xiaoqing River is clear. Grade III water corresponds to conservation district of drinking water, areas for fish over wintering and migration as well as swimming areas. Huanxiangdian, Damatou and Xinfeng Zhuang are located downstream of Muli gate about 25 km, 37 km and 65 km away, respectively. The annual average values of CODcr, BOD_5 and ammonia nitrogen of the three monitoring sites do not meet the demands of Grade V of the "Environmental Quality Standard for Surface water (GB3838-2002)"(State Environmental Protection Administration of China 2002), of which the values of ammonia nitrogen exceeded the limit greatly, and shows that water in the three sites was heavily polluted. Grade V water corresponds to the water for agriculture and general landscape, without exposure to the human body. The quality values of water in excess of limit values of Standard V means that water is polluted.

The four sites, Muli gate, Huanxiangdian, Damatou and Xinfeng Zhuang are very different in water quality, from unpolluted to polluted, but alligatorweed was found in all sections of the upper Xiaoqing River, which indicates that the differences in water quality did not affect its distribution.

Parameters of Alligatorweed

The length of main branches of alligatorweed measured in the Peng Zhuang's population in the upper Xiaoqing River ranged from 198 cm to 382 cm, with an average value of 266.67 ± 24.01 cm. The number of nodes on the main

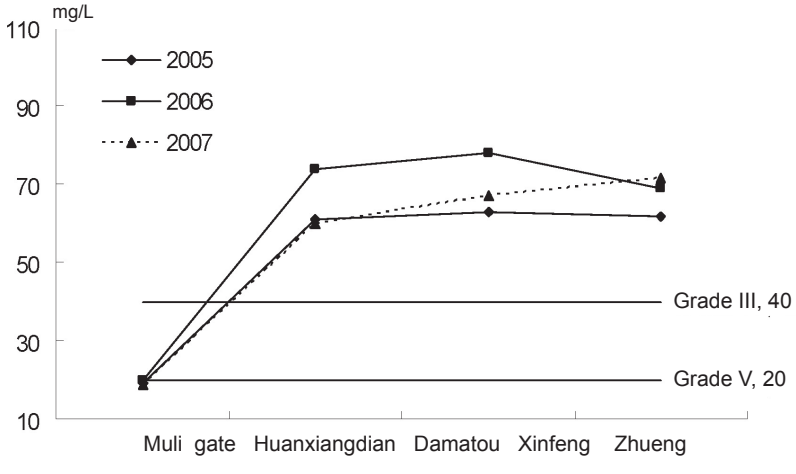

Figure 3. A: The values of chemical oxygen demand (CODcr) of the four monitoring sites of Muli gate, Huanxiangdian, Damatou and Xinfeng Zhuang in the upper Xiaoqing River. The limit values of Grade III and Grade V of "Environmental Quality Standard for Surface Water (GB3838-2002)" (State Environmental Protection Administration of China 2002) are also shown. B: A picture of alligatorweed at the main collection site at Peng Zhuang that near Muli gate growing in the clean waters of the upper Xiaoqing River.

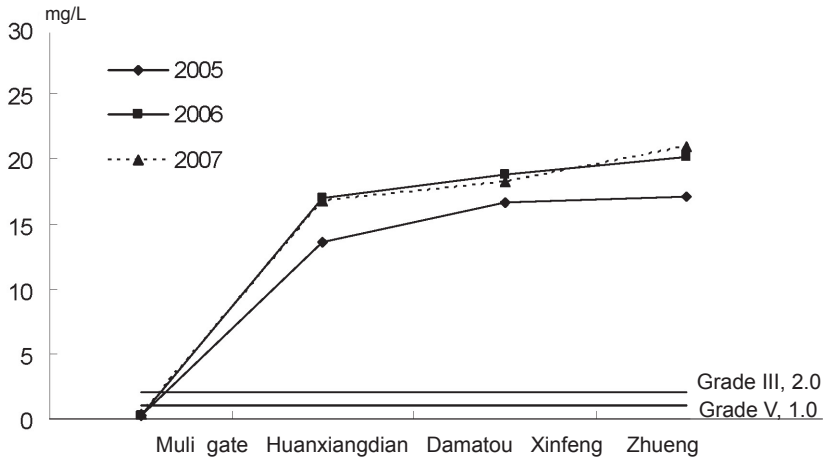

Figure 4. A: The values of ammonia nitrogen of the four monitoring sites of Muli gate, Huanxiangdian, Damatou and Xinfeng Zhuang in the upper Xiaoqing River. The limit values of Grade III and Grade V of "Environmental Quality Standard for Surface Water (GB3838-2002)" (State Environmental Protection Administration of China 2002) are also shown. B: A picture of alligatorweed nearly covering the Zhaowanghe a small flood discharge and irrigation waterway between the Xiaoqing River and the Yellow River in eastern Jinan. This site is between the Huanxiangdian and Damatou monitoring sites and is heavily polluted.

branches was 22-33 with an average of 27.01 ± 2.25. The number of adventitious roots per main branch was 13-25 with an average of 17.11 ± 0.84. The number of branches from each main branch ranged from 3 to 9 with an average of 5.71 ± 0.62, with most secondary branches having additional tertiary branches. The number of flowers in each main branch and its branches was 3-10 with an average of 6.52 ± 0.61.

The number of nodes, adventitious roots and secondary branches of the longest main branch was 30, 25 and 7, respectively. The longest secondary branch was 185 cm in length, and the number of its nodes, adventitious roots and tertiary branches was 20, 13 and 3, respectively. The number of main branches per linear meter ranged from 376-511, with an average of 436.52 ± 55.33.

Hydrochory is an important means of propagule transport for plants (Nisslon et al. 2010), and dispersal by fragmentation is particularly effective for aquatic plants (Riis and Sand-Jensen 2006). The stems of the alligatorweed are hollow, buoyant, and easily broken (Julien et al. 1992), which contributes to their dispersal ability and the invasiveness of this species in aquatic environments. The invasiveness of plants has a close relationship with their reproductive ability (Barret 1983), and alligatorweed can reproduce by asexual means and can grow into a new plant from a short stem (Lin and Qiang 2004).

The population of alligatorweed in the upper Xiaoqing River has a very high likelihood of invading the middle and lower Xiaoqing River, and also has a likelihood of invading other rivers, for example Yellow River, that have hydro connections with Xiaoqing River. The updates and revision for the previous distribution map, and certain basic characterization of alligatorweed stands in the upper Xiaoqing River will be helpful to understand and predict the status and spread of the alligatorweed population in northern China, and also helpful to make a reasonable management strategy for the weed in northern China.

Local Impacts

Alligatorweed was found to have invaded two of the five quadrants of vegetable land surveyed, however the infestations were not severe and the local farmers did not consider the weed to have damaged vegetable production or to be a problem weed. No alligatorweed was found in any of the rice production areas or in fish ponds in the upper Xiaoqing River. Indeed, without special introduction, most local farmers were not familiar with it.

The main impact of the weed in the upper Xiaoqing River is the restriction of flood flow in the rainy seasons. Since 2005, the management office of the Xiaoqing River of Jinan removes and controls this weed in waterways by mechanical methods, and the flood discharge is no longer a problem.

Although alligatorweed was not found to cause serious damage to agriculture in the upper Xiaoqing River, the losses are significant in south China. The results show that the weed reduces vegetable production by 5% to 15% on average, and may cause losses over 20% (Yin 1992). It can reduce yields in sweet potato and rice by 63% and 45% respectively (Liu and Huang 2002). Over 200 hectares of fish ponds were abandoned due to alligatorweed infestations in the neighboring suburbs of Chongqing city (Zhang et al. 1993).

The agriculture of Shandong plays an important role in China. The output of grain crops of the province account for 8.27% of the national total output, and rank second in China (Ministry of Agriculture of China 2007). Areas along the Yellow River and Xiaoqing River are the main regions for agriculture in Shandong. Presently, no obvious negative agricultural impact was found in this study, however the presence of well-established populations in the region warrant both weed control efforts and education of local populations to the potential agricultural impacts and weed control measures in Shandong, and other provinces of northern China.

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