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Regular research paper

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THE ECOLOGICAL PROFIT IN PHENOLOGY AND POLLINATION OF ALIEN SPECIES *SPARTINA ALTERNIFLORA* (LOISEL) INTRODUCED TO COASTAL WETLANDS IN NORTHERN CHINA

ABSTRACT: For the plant species, effective phenology and pollination syndromes can increase viable seeds which enhance the species to colonize new areas. So the species with a suit of advantageous characteristics inherent in its reproductive ecology is critical to its dispersion. *Spartina alterniflora* – a perennial grass native to coastal marshes of America was introduced to China in 1979 and now it is distributed in the Tianjin coastal wetlands of northern China. Flowering phenology was examined and indicated that in northern localities, flowering initiated in mid-August and senescence occurred by the end of October. The pollen-ovule ratio was 9875 (± 2150). Abundant pollen grains compensated for a lack of pollen viability. Pollen viability was 10% approximately when pollen released 4 hours, but stigma receptivity was above 10% when stigmas exerted 7 hours. Stigmas maintained viability longer than the period of pollen release, ensuring successful pollination. Scanning electron microscopy was used to observe stigma surface characters and pollen viability. Further investigation proved stigma adaptations to increase pollen capture and exterior pollen tube growth following pollination was beneficial to fertilization. The results suggested that flowering phenology and pollination traits are vital to pollination and production of viable seed, enabling the species to spread rapidly and succeed in new habitats of northern China.

KEY WORDS: adaptation, florescence, introduced species, pollination, sexual reproduction, *Spartina alterniflora*

1. INTRODUCTION

Spartina alterniflora Loisel (smooth cordgrass), a perennial grass native to the coastal marshes of eastern America, was introduced to Luoyuan Bay (26°05'N, 119°55'E), Fujian Province in China for the purposes of erosion control in December 1979 (Xu and Zhuo 1985). Over the last two decades, the species has become established in coastal wetlands from Beihai (21°05'N, 109°12'E) of Guangxi Province in southern China to Haihe estuary (39°03'N, 117°45'E) of Tianjin in northern China. The use of this introduced grass in coastal reclamation, restoration efforts and pollution control has received increasing attention due to its high stress tolerance, rapid growth rate and colonizing ability in China's intertidal zones (Song 1997, Zhu and Qin 2003, Cao *et al.* 2005, Chen *et al.* 2005). Since its introduction to China, *S. alterniflora* has been demonstrated to impede shoreline erosion. Therefore, the species plays an important role in estuarine ecology and contributes to the integrity of salt marsh

ecosystems. In contrast to the ecological benefits of the species, the establishment of *S. alterniflora* in the tidal marshlands of Tianjin north China, *S. alterniflora* has displaced native species and is presently the sole dominant member of the community owing to opportunist traits.

S. alterniflora dominates tidal wetland salt marsh communities due to its effective propagation abilities and subsequent competitive advantage. The geographic extent of *S. alterniflora* is much greater in China than the other regions of the world (Deng *et al.* 2006). For example, the species has also been reported from Luoyuan Bay, Fujian Province (Xu and Zhuo 1985) where the species flowers from July to October. In Chongming Dongtan, Shanghai, the species demonstrates a lower seed set (*i.e.* lower percentage of filled seeds over seeds on one panicle) (Zhang *et al.* 2006). In addition, *S. alterniflora* has successfully established in parts of North America, outside its native range. Notably, the species is documented in the salt marshes of South San Francisco Bay. At this locality, *S. alterniflora* produces copious pollen of high fertility and successfully hybridizes with *S. foliosa*, a species native to the San Francisco Bay estuaries (Antilla *et al.* 1998). In Louisiana salt marshes, data regarding fertilization and seed set under both self- and cross-pollination (Fang *et al.* 2004) reveals that pollination characteristics are critical in understanding the invasive properties of the species.

For success, an introduced plant species must possess a suite of advantageous characteristics inherent in its reproductive ecology, such as effective phenology and pollination syndromes, and increased seed germination rates (Wang *et al.* 2006). Pollen viability duration, sexual resource allocation and pollination characteristics are integral to high rates of pollination and subsequent fertilization (Pacini *et al.* 1997). In addition to the plant breeding system characterized in the species (Cruden 1977), the hermaphroditic sexual resource allocation (Preston 1986) is reflected in the pollen/ovule ratio availability. In anemophilous plants, stigma morphology increases effective pollen grain capture and germination, which ensures fertilization (Wang 2001). However, little information of

the reproductive ecology of *S. alterniflora* is reported from northern China.

In order to elucidate the species' adaptive plasticity to dominate habitats in north China, we studied *S. alterniflora*'s phenology and pollination characteristics at the Tianjin, northern China salt marsh. The results of this study led to an enhanced understanding of the species capacity to colonize new areas; which can be extrapolated to other invasive species adaptations to non-native habitats.

2. STUDY SPECIES AND SITE

S. alterniflora is a wind-pollinated grass with protogynous flowers. In protogynous flowering, stigmas exert before anther dehiscence in the same floret. Following the flag leaf emergence from the sheath, the panicle begins to exert stigmas, which proceeds from the upper one third of the panicle and progresses simultaneously toward the upper and lower panicle. *S. alterniflora* flowers with variable flowering dates throughout its geographic distribution. Therefore, flowering is an important determinant influencing seed set and vegetative reproduction. The Tianjin coastal wetland (38°33'N–40°14', 116°42'–118°03'E) (Cao *et al.* 2005) was chosen as the study site due to the high frequency of cordgrass. Three zones were delimited within the marsh and indicated by permanent tags as follows: (1) the low edge of the marsh; (2) 20 m inland from the marsh edge; and (3) 40 m inland from the marsh edge.

3. STUDY METHODS AND DATA ANALYSIS

The study was initiated in August and terminated in November 2005. The date of the first panicle emergence from the sheath was referred as the flowering date. Flowers reached anthesis in August and flowering phenology was recorded twice a week until senescence in October. During the flowering period, 30 florets were sampled from each intertidal zone indicated above. Pollen grains were collected when anthers shed pollen at 10:00. Pollen viability was measured from 10:00 to 18:00 at intervals of two hours and stigmas were collected starting from the time of exertion at two hours intervals. Pollen vi-

ability and stigma receptivity were measured using MTT method (Dafni 1992). The pollen and stigma samples were fixed respectively in 0.5% MTT sucrose solution for one hour. The samples turning into dark blue indicated they were viable, otherwise the samples' viability was lost. Pollen-ovule ratios were determined according to Cruden (1977). Pollen and ovule quantity of each floret was calculated, respectively. Pollen-ovule ratio was the ratio of pollen to ovule in the same floret. In November, 30 samples were collected to investigate reproductive traits from each zone and means subsequently generated.

Scanning electron microscopy (SEM) was employed to investigate stigma characteristics that ensured pollen grain capture and pollen grain germination capability. Ten panicles were bagged before stigma exertion in each zone. Pistils of each floret were taken from the inflorescence prior to pollination, and hand pollinated at 15 min intervals following pollen shed, 10:00 to 12:00. Pistils were fixed in 3% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2), treated for eight hours under a vacuum, and subsequently washed three times for 20 min each in 0.1 M sodium phosphate buffer. Samples were post fixed at room temperature in the dark for three hours in 1% aqueous osmium tetroxide (OsO_4), washed twice for 20 min each in sodium phosphate, and dehydrated through a graded series of ethanol. When the samples reached critical point dried, they were sputter coated with gold and viewed in a FEI Quanta SEM operated at 20.0 kV. Pollen grains located on the stigma under the open pollination were observed with a stereomicroscope.

The descriptive statistics of reproductive trait was evaluated with Excel 2003. The correlation between the variation of pollen vi-

ability or stigma receptivity and the time was analyzed using Origin 7.5.

4. RESULTS

Flowering initiated in the middle of August and terminated by late October, resulting in a 2.5 month flowering period. Eighty-five to ninety percent of all plants flowered between early September and early October. Stigmas exerted from the florets approximately two days prior to pollen shed in the same floret. Stigmas were viridescent and receptive upon exertion from the floret. Whether or not pollination occurred, stigmas changed color from viridescent to brown and shriveled gradually following eight hours of exertion (Fig. 1A). Anthers shed pollen from approximately 10:00 to 14:00 with a two hours maximum pollen shed period. The pollen-ovule ratio was 9875 ± 2150 (Table 1). *S. alterniflora* caryopses (hereafter called seeds) were observed from September to November, with most maturing in the latter month. Long panicles developed with an average length of 27.5 ± 2.5 cm. Each panicle averaged 236 (± 38) seed and an average seed set of 67% ($\pm 2.4\%$) with ranging from 61% to 75%. A single plant produced up to 169 (± 31) viable seeds per year (Table 1).

Pollen viability presented an exponential downtrend from the time of pollen release. The highest viability was 33% ($\pm 2.9\%$) when pollen began to shed and decreased to 2.8 ($\pm 0.9\%$) as pollen released for eight hours (Fig. 2). The receptive stigmas were plump (Fig. 4C) with abundant pollen grains (Fig. 4A). Stigma receptivity presented a linear downtrend from the time of stigma exertion. All stigmas were receptive when they exerted and the receptivity decreased to $0.6 \pm 3.9\%$ following eight hours of exertion (Fig. 3).

Table 1. Mean (\pm S.D.) values of reproductive traits for *S. alterniflora* in Tianjin costal wetland (n = 90).

Panicle length (cm)	27.5 \pm 2.5
Pollen/ovule ration	9875 \pm 2150
Seed wt/panicle (g)	1.07 \pm 0.19
Kernel wt/1000kernel (g)	6.31 \pm 0.14
Total seeds/panicle (no)	236 \pm 38
Filled seeds/panicle (no)	169 \pm 31
Seed set (%)	67 \pm 2.4

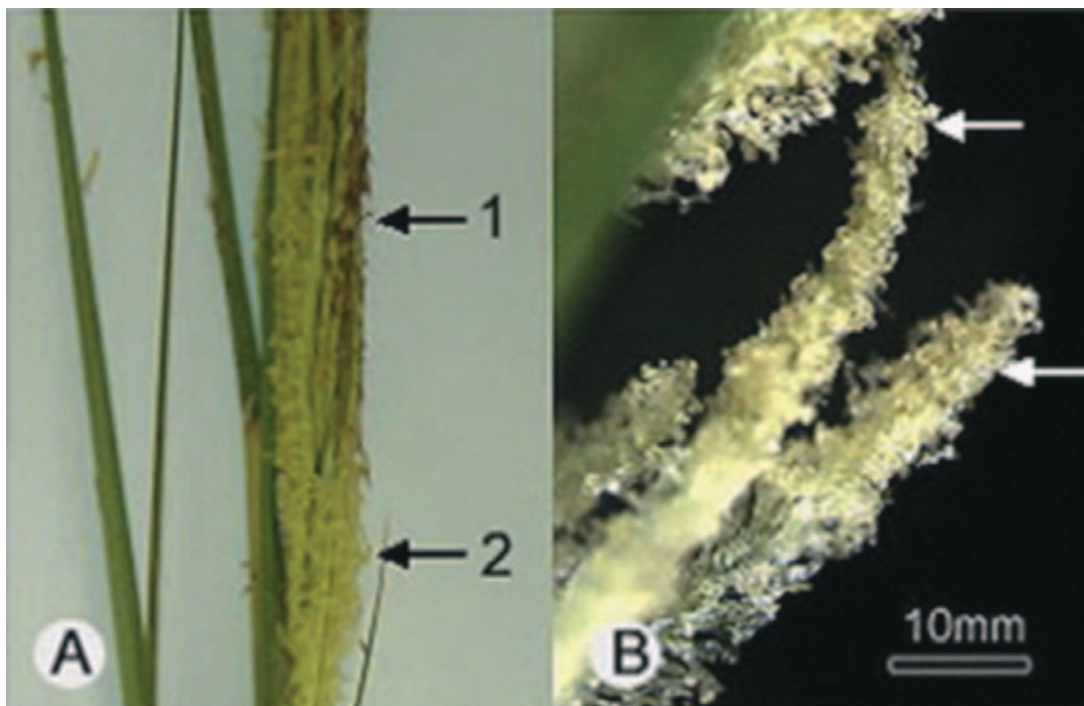


Fig. 1. The stigma receptivity and pollen grains on the stigmas for *S. alterniflora*. A: The stigma lost receptivity (arrow 1), the receptive stigma (arrow 2). B: Abundant pollen grains adhered on the stigmas (arrows).

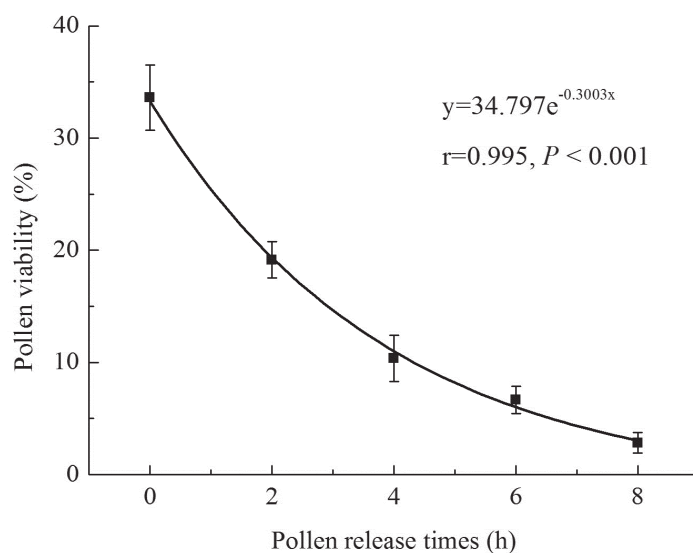


Fig. 2. Correlation between pollen viability and pollen release time for *S. alterniflora* (n = 90).

Abundant pollen grains adhered on the stigmas (Fig. 1B). Pollen grains germinated immediately after contact with the stigmas (Fig. 4A). Abundant regular plump papillae were observed on receptive penniform stigmas (Fig. 4B) and pleat spread on the papillae (Fig. 4C). Pollen grains and papil-

lae were plump at the onset of pollination (Fig. 5A, B). Pollen grains germinated randomly on the stigmatic papillae or at a locating position (Fig. 5A–I). Subsequently, pollen tubes elongated enough to penetrate stigmatic papillae (Fig. 5A, C, D), ensuring successful pollination. As the pollen sub-

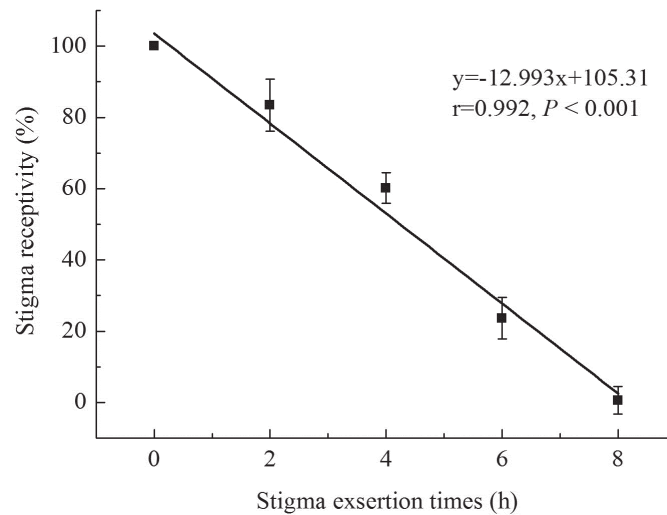


Fig. 3. Correlation between stigma receptivity and stigma exertion time for *S. alterniflora* (n = 90).

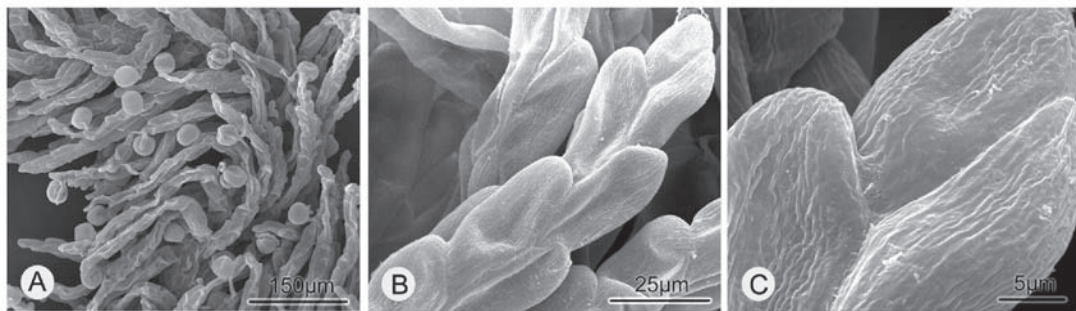


Fig. 4. The stigma characteristics resulting in effective pollen grain capture in *S. alterniflora*. A: Simultaneous pollen grain germination of the stigmas. B: Regular plump papillae arranged on the penniform stigmas. C: Abundant pleat spread on the plump papillae.

stance was released into the pollen tubes, the pollen wall wrinkled, and the stigma withered (Fig. 5C–H). Finally, pollen grains and stigma papillae shriveled (Fig. 5I).

5. DISCUSSION

Success of an introduced species lies in its pre-adaptation to a new environment. In this study, *S. alterniflora* initiated flowering in August through October. *S. alterniflora* demonstrated a different phenology throughout its geographic distribution, suggesting rapid adaptation to northern China. A long stigma exposure period before pollen release might favor cross-pollination, increasing the levels of genetic diversity. Enhanced genetic variability in *S. alterniflora* may be its most important means of adaptation to new environ-

ments (Wang *et al.* 2006). Recent research indicated that in plant breeding systems, the time between receptive stigmas and pollen release is as important as the types and frequency of pollinating insects (Liu *et al.* 1997, 1998, He and Liu 2003). In *S. alterniflora*, the stigmas were receptive immediately after exertion and remained viable past pollen release, ensuring successful pollination. Pollen viability and pollen-ovule ratio results indicated that *S. alterniflora* compensated for the lack of pollen viability *via* the production of abundant pollen grains. The large number of pollen grains located on the stigmas suggested increased competition among pollen grains. Therefore, more fit offspring could be generated (Marshall and Whittaker 1989). However, late exerting stigmas could be self-pollinated from the top anthers in the

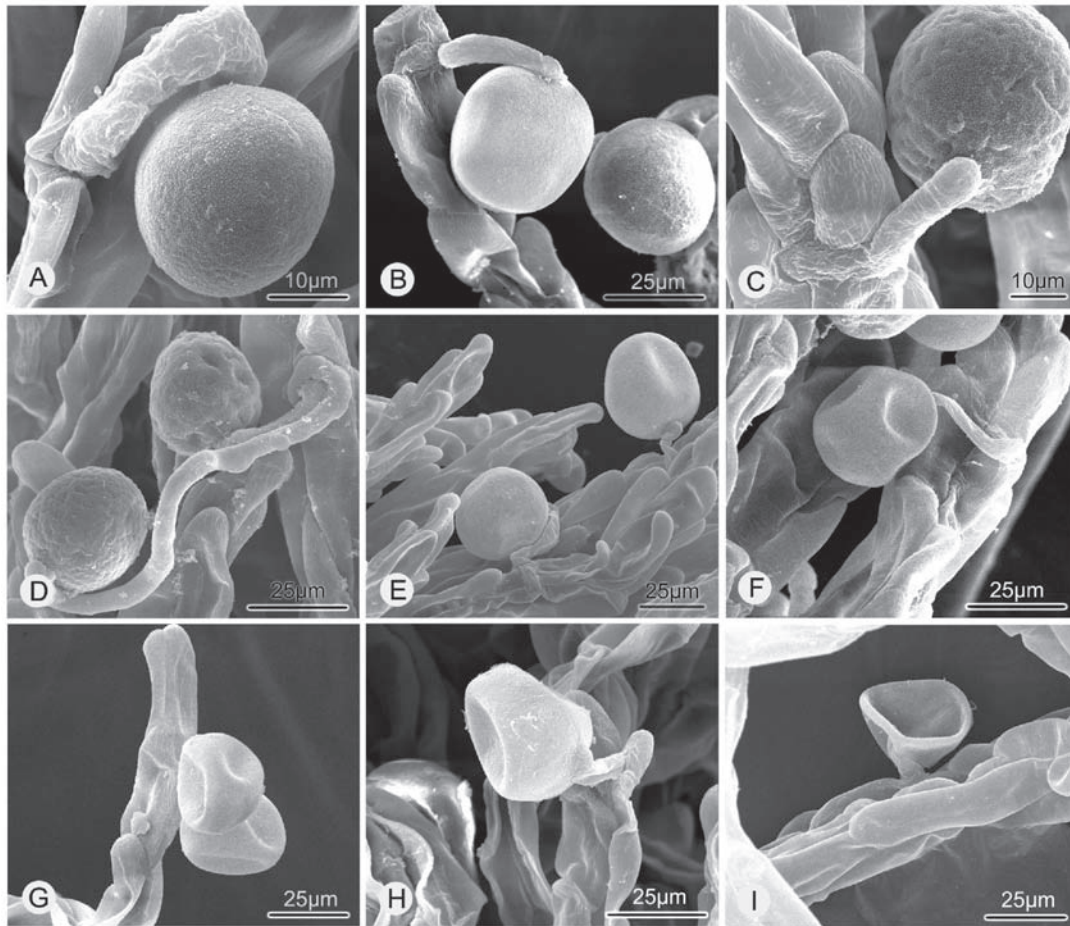


Fig. 5. Pollination and the pollen grain germination location on the papillae of *S. alterniflora*. A and B: 0.25 h after pollination. C, D, E: 0.5 h after pollination. F, G, H: 1 h after pollination. I: 1.5 h after pollination. A, C, D: Showing the extended exterior high viability of pollen tubes.

same inflorescence. Inbreeding depression from self-pollination among clones is low, whereas it is beneficial to low self-fertility clone dispersal (Daehler 1998). The anthesis phenology in *S. alterniflora* suggested that the inflorescence was largely cross-pollinated by the surrounding flowers, even though the clones continuously expanded. Therefore, viable pollen was not a limitation for seed set.

In plants, floral syndromes have co-evolved with pollination patterns (Guo and Huang 1999). In *S. alterniflora*, the regular plump papillae on penniform stigmas (Fig. 4B) and the spreading pleat (Fig. 4C) enlarged the pollen receptive area. A stigmatic adaptation to capture more pollen grains would be of great benefit to pollen loading (Fig. 4A). In anemophilous plants, pollen grains are randomly located on the

stigmas, a mechanism for successful wind pollination. Exterior prolonged pollen tubes on the stigmas indicated a high capability of pollen germination (Fig. 5A, C, D). Pollen grains maintained high viability in the first two hours of pollen shedding, a period of time longer than that required for pollination. The viable pollen grains and exterior pollen tube growth ensured successful fertilization.

This study demonstrated that the pollination traits of *S. alterniflora* are advantageous to the sexual reproduction and the flowering phenology is well adapted to environmental conditions in northern China. This is likely one explanation for the rapid range extension and proliferation of *S. alterniflora* and subsequent alteration of native plant communities.

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6. REFERENCES

- Anttila C.K., Daehler C.C., Rank N.E., Strong D.R. 1998 – Greater male fitness of a rare invader (*Spartina alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridization – *Amer. J. Bot.* 85: 1597–1601.
- Cao D.Z., Wang Y.S., Zhang D.R., Gu L.J., Tang T.G. 2005 – Application of *Spartina alterniflora* on blow-fill-construct sea wall engineering – *Engineering Science*, 7: 14–23 (in Chinese with English abstract).
- Chen Y., Zheng H.L., Xiao Q., Huang W.B., Zhu Zh. 2005 – Effects of salinity on oxidative and antioxidative system of *Spartina alterniflora* – *J. Xiamen Univ. (Nat. Sci.)*. 44: 576–579 (in Chinese with English abstract).
- Cruden R.W. 1977 – Pollen-ovule ratios: A conservation indicator of breeding systems in flowering plants – *Evolution*, 31: 32–46.
- Daehler C.C. 1998 – Variation in self-fertility and the reproductive advantage of self-fertility for an invading plant (*Spartina alterniflora*) – *Evol. Ecol.* 12: 553–568.
- Dafni A. 1992 – *Pollen ecology, a practical approach* – New York: Oxford Univ. Press, pp. 1–57.
- Deng Z.F., An Sh.Q., Zhi Y.B., Zhou Ch.F., Chen L.Zh., Cong J., Fang Sh.B., Li. H.L. 2006 – Preliminary studies on invasive model and outbreak mechanism of exotic species, *Spartina alterniflora* Loisel. – *Acta. Eco. Sin.* 26: 2678–2686 (in Chinese with English abstract).
- Fang X.B., Subudhi P.K., Venuto B.C., Harrison S.A. 2004 – Mode of pollination, pollen germination, and seed set in smooth cordgrass (*Spartina alterniflora*, Poaceae) – *Int. J. Plant. Sci.* 165: 395–401.
- Guo Y.H., Huang Sh.Q. 1999 – Evolution of pollination system and characters of stigmas in Najadales – *Acta. Phytotax. Sinica.* 37: 131–136 (in Chinese with English abstract).
- He Y.P., Liu J.Q. 2003 – A review on recent advances in the studies of plant breeding system – *Acta Phytoecol. Sin.* 27: 151–163 (in Chinese with English abstract).
- Liu L.D., Wang Zh.L., Tian G.W., Shen J.H. 1997 – Studies on sexual reproduction and vegetative propagation of *Eleutherococcus senticosus* (Araliaceae) – *Acta. Phytotax. Sin.* 35: 7–13 (in Chinese with English abstract).
- Liu L.D., Wang Zh.L., Tian G.W., Shen J.H. 1998 – The development of gynoeceum after anthesis and fertilization in *Eleutherococcus senticosus* (Araliaceae) – *Acta. Phytotax. Sin.* 36: 111–118 (in Chinese with English abstract).
- Marshall D.L., Whittaker K.L. 1989 – Effects of pollen donor identity on offspring quality in wild radish, *Raphanus sativus* – *Amer. J. Bot.* 76: 1081–1088.
- Pacini E., Franchi G.G., Lisci M., Nepi M. 1997 – Pollen viability related to type of pollination in six angiosperm species – *Ann. Bot.* 80: 83–87.
- Preston R.E., 1986 – Pollen-ovule ratios in the Cruciferae – *Amer. J. Bot.* 73: 1732–1740.
- Song L.Q. 1997 – *Spartina alterniflora* and role of coastal protection – *East. Chin. Sea. Ocean.* 15: 11–19
- Wang X.F. 2001 – The floral syndromes adapted to pollination patterns of four genera in Alismataceae from China, – *J. Wuhan Univ (Nat. Sci. Ed.)*, 47: 485–492.
- Wang Q., An Sh.Q., Ma Zh.J., Zhao B., Chen J.K., Li B., 2006 – Invasive *Spartina alterniflora*: biology, ecology and management – *Acta. Phytotax. Sin.* 44: 559–588.
- Xu G.W., Zhuo R.Z. 1985 – Preliminary studies of introduced *Spartina alterniflora* Loisel in China – *J. Nanjing Univ. (Research advanced in Spartina – achievements of past 22 years)*, pp 212–225.
- Zhang D., Yang M.M., Li J.X., Chen X.Y. 2006 – Vegetative dispersal ability of *Spartina alterniflora* in eastern end of Chongming Island – *J. East. Chi. Nor. Uni.* 2: 130–135.
- Zhu X.J., Qin P. 2003 – The alien species *Spartina alterniflora* and Spartina eco-engineering – *Mar. Sci.* 27: 14–19.

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