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THE EFFECT OF A FOREIGN POLLEN ON OVULE DEVELOPMENT IN *DIERVILLA LONICERA* (CAPRIFOLIACEAE)

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SUMMARY

When pollen from *Hieracium floribundum* Wimmer and Grab. (Compositae) was applied to stigmas of *Diervilla lonicera* Mill. (Caprifoliaceae) in mixtures with *Diervilla* pollen, *Diervilla* fecundity was strongly depressed. While this phenomenon is unlikely to be of importance to *Diervilla* under field conditions, this property of *Hieracium* pollen may have stronger effects on other ecological associates. The only other reported instance of inhibitory pollen is from *Parthenium hysterophorus* L., which, like *Hieracium*, has allelopathic properties.

INTRODUCTION

The deposition of heterospecific pollen on stigmas has been considered a component of competition between plant species populations for the effective service of inconstant pollinators (Waser 1978a, b; cf. Levin and Anderson, 1970; Wissel, 1977). These treatments have concentrated on indirect negative effects of foreign pollen, such as the displacement of proper grains from stigmas of limited area and the pollen wastage occasioned by deposition on the wrong species. However, direct involvement of foreign grains in the complicated biochemistry of the stigma is also a possibility. With the exception of Sukada and Jayachandra's (1980) recent report of 'pollen allelopathy' in *Parthenium* (Compositae), the direct effect of foreign grains on fertilization appears to have received limited experimental attention, although the literature concerning effects of conspecific pollen grain number and dilution provides some parallels, e.g. Brewbaker and Majumder (1961), Hornby and Charles (1966) and Jennings and Topham (1971). This paper describes the analysis of the functional response of ovule development in *Diervilla lonicera* Mill. (Caprifoliaceae) to the proportion of foreign pollen (from *Hieracium floribundum* Wimmer and Grab., Compositae) applied in hand-pollinations. The floral biology of *D. lonicera* is described by Schoen (1977).

MATERIALS AND METHODS

The *Diervilla* plants grew along spruce–fir forest edges on the Jonah property, 5 km southeast of Doaktown in central New Brunswick, Canada. *Hieracium floribundum* was an abundant weed of the adjacent open areas, flowered at the same time (early July), and was visited by the same bumble bee species (most commonly,

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Bombus terricola Kirby and *B. vagans* F. Smith) (Schoen, 1977; Thomson and Plowright, 1980; Thomson, 1981). A few flights between *Hieracium* and *Diervilla* by individual foragers were observed, and several bees were captured carrying mixed loads of pollens, but this inconstancy was rare, which is not surprising considering the dissimilarity of the two flowers and of the techniques required to extract nectar and pollen from them. *Hieracium* was chosen as a heterospecific pollen diluent more for its convenience than from any conviction that visitors regularly moved between the two species; *Diervilla* was chosen as a pollen recipient because it has a manageable ovule number (about 30), is easily emasculable and bears a large and accessible stigmatic surface.

Diervilla flowers were emasculated shortly before natural bud break and enclosed in bags of nylon mesh to exclude visitors. Pollinations were performed from 30 June to 8 July 1979 and from 5 to 11 July 1980 on fully open, receptive flowers. Pollen preparation involved mixing fresh pollen from several *Diervilla* flowers and several *Hieracium* heads by vigorous stirring in a vial with a fine paint brush. Repeated samples from such mixtures showed little variation in the species ratio of the grains. The mixture was applied to receptive *Diervilla* stigmas by the same brush, depositing a 'saturation' number of grains far in excess of the number of ovules. As a small test of the replicability of pollen application, three stigma-loads were removed immediately after application by pressing the stigmas into adhesive cubes of basic fuchsin-tinted glycerine jelly (Beattie, 1971a) and preparing slides to count the grains of each species. The grains are easily distinguished by size or structure, the *Hieracium* being smaller and more echinate. The populations recovered from these three stigmas were [number of *Hieracium* grains:number of *Diervilla* grains (fraction *Hieracium*)]: 130:177 (42 %); 95:130 (42 %); 129:186 (41 %). The proportional composition was thus remarkably consistent, although the total numbers applied varied. The same glycerine jelly technique was used to mount samples of the various mixtures to determine the ratios being applied. The samples were applied to jelly blobs in the same way in which they were brushed on to the stigmas. After pollination, flowers were rebagged until harvest when developed and aborted ovules could be easily counted by dissection of the developing fruits.

In 1979, nine experimental mixtures, ranging from 13 to 93 % *Hieracium*, were applied to 77 *Diervilla* flowers. Unfortunately, no pollinations were performed with pure *Diervilla* pollen to provide an uncontaminated control. This defect was remedied in 1980, when treatments of 0, 5, 10, 20, 40 and 60 % *Hieracium* were applied. The 1980 treatment levels must be regarded as approximate; the mixtures were adjusted to be as close to the specified percentages as practical, but the actual test slides and the grain count data were accidently destroyed. In both years, all *Diervilla* pollen was taken from the same donor clone and the same recipient clones were used. This ensured that the observed responses were not confounded by different compatibility relationships.

RESULTS

The results are given in Figure 1. There is a striking decrease in *Diervilla* ovule development as *Hieracium* grain proportion increases, amounting essentially to complete reproductive failure for all *Hieracium* proportions above 50 %. The presence of even a small amount (5 to 10 %) of *Hieracium* pollen is sufficient to depress development greatly in comparison to pure-pollinated controls. The high

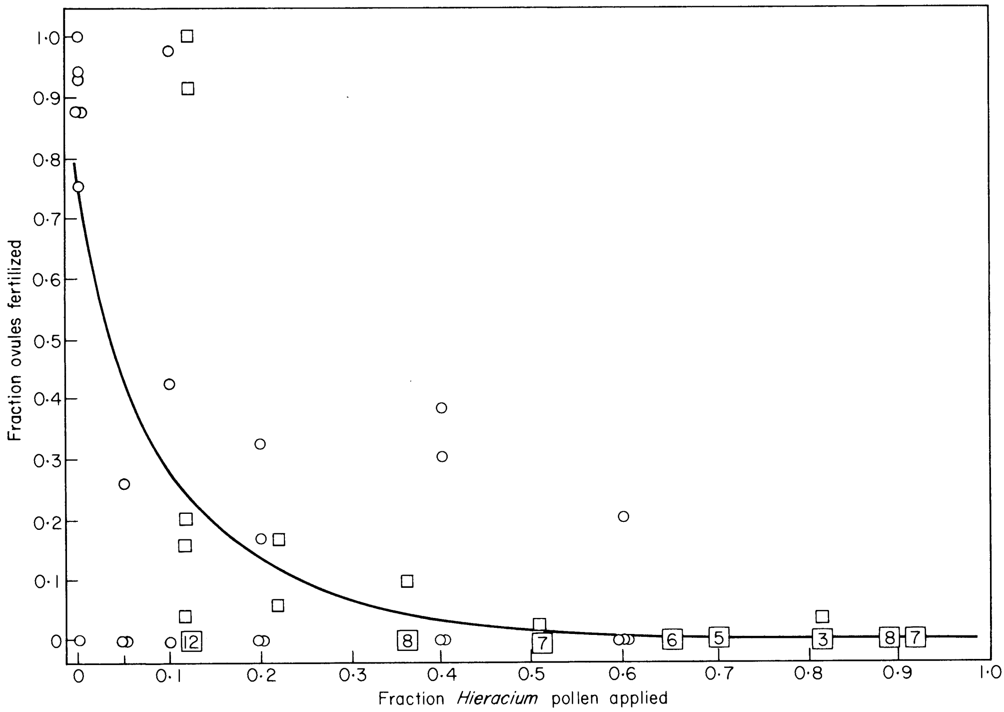

Fig. 1. Results of artificial pollinations of *Diervilla lonicera* with various mixtures of *D. lonicera* and *Hieracium floribundum* pollen. The treatment levels, which must be regarded as approximate for the 1980 data (see text), are computed according to numbers of grains, not weight or volume. Large squares containing a number indicate several coincident points of zero fertilization. The curve was fitted by eye. □, 1979; ○, 1980.

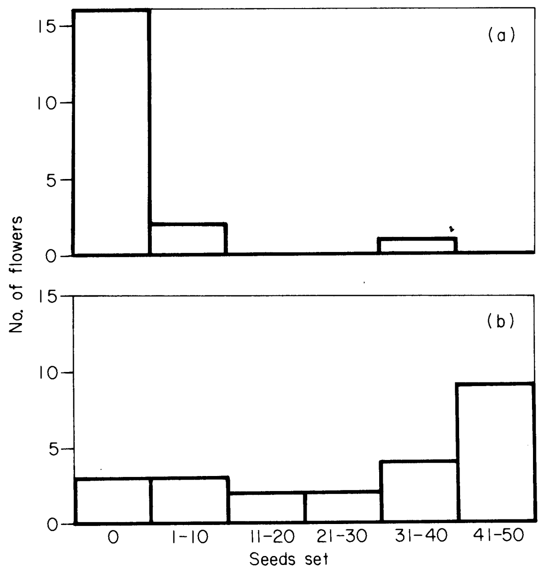

Fig. 2. Results of artificial (a) self- and (b) cross-pollinations on *Diervilla lonicera*, indicating probable self-incompatibility. Field data: 1979.

success rate of the controls (Fig. 1) indicate that the *Diervilla* donor and recipient clones were compatible.

The results of self- and cross-pollinations summarized in Figure 2 suggest that *D. lonicera* is self-incompatible, which concurs with Schoen's (1977) conclusions on *D. lonicera* in Michigan. Some of the fertilizations in the selfed flowers may reflect pollen transfer by minute staphylinid beetles which occasionally got through the mesh bags.

DISCUSSION

Functional response of ovule development to foreign pollen

The simplest explanation for a reduction in ovule development with an increase in the proportion of heterospecific grains would be direct physical displacement of conspecific grains, implying competition between the two grains for a limited stigmatic area but assuming that pollen germination and growth are unaffected. This mechanism, one of several components of plant competition modelled by Waser (1978a), should yield a roughly linear increase in fertilization with proportion of correct pollen. The strong depressive effect of moderate concentrations of *Hieracium* noted in Figure 1, however, suggests that surface area considerations alone provide insufficient explanations; other factors must be complicating the interaction.

One such factor may be the 'pollen population effect' described by Brink (1924) in which the percentage germination of a population of grains *in vitro* is a direct function (linear in *Petunia*, Brewbaker and Majumder, 1961) of the population size. Brewbaker and Majumder (1961) consider the appearance of this mutual stimulation of grains in many unrelated genera to imply 'universality of the phenomenon', and attribute its action to a water-soluble growth factor present in pollen. Such an effect, if it operated on stigmas of *Diervilla*, obviously could contribute to the non-linearity evident in Figure 1. Whether this effect, combined with simple reduction in pollen population size, is sufficient to explain the observed *amount* of non-linearity is uncertain.

The results of Jennings and Topham (1971) on *Rubus*, using heat-killed conspecific pollen as a diluent, show a much less extreme (i.e. more linear) effect of pollen dilution on percentage drupelet set than the response observed here. This raises the possibility that *Hieracium* grains do not act as a neutral diluent, simply taking up stigmatic area and diluting *Diervilla* pollen growth factors, but that they inhibit germination or growth of *Diervilla* grains by active means, possibly in a manner analogous to normal incompatibility reactions. Firm demonstration of active effects must await the investigation of the functional response of *Diervilla* fertilization to varying amounts of its own pollen so that the role of the pollen population effect may be assessed.

The nature of normal pollen–stigma interactions in *Diervilla* must also be studied before the disruption of the system by foreign pollen can be understood. Such investigations were beyond the scope of this field study, and a survey of de Nettancourt's (1977) monographic review reveals little information about incompatibility in the Caprifoliaceae. *Diervilla* appears to have a 'wet' stigmatic surface of the type in which incompatibility proteins are released from pollen into the stigmatic fluid; it is quite likely that substances from the *Hieracium* pollen would also be released, and the inhibitory effect of the foreign pollen may be expressed at the stigma. The modification of stigmatic receptivity to one type of grain by another type is well known in the 'mentor effect', where, within a species, killed compatible pollen can stimulate the growth of incompatible pollen (de Nettancourt, 1977). In our experiments, we find a reduced, rather than increased, acceptance of pollen, but the site and mechanism of the reaction may be analogous to the mentor effect.

Active inhibition has been reported for pollen of the weedy *P. hysterophorus* L. by Sukada and Jayachandra (1980). *Parthenium* pollen not only inhibits seed set in several test species when applied to stigmas; it also inhibits pollen germination and tube growth of other species in artificial cultures of mixed pollens, and can even reduce the chlorophyll content of foliage on which it is deposited. Aqueous pollen leachate shows similar inhibitory properties. Sukada and Jayachandra imply that the toxicity of the pollen is related to the generally allelopathic nature of the plant, which also releases soluble growth inhibitors through root exudates, foliar leaching and volatilization. This seems to parallel the situation in *Hieracium*, another pernicious composite weed with reported antibiotic potential (anecdotal evidence cited by Curtis, 1959, pp. 316–318 and Guyot, 1957). Sukada and Jayachandra suggest that the toxicity of *Parthenium* pollen may inhibit reproduction of its neighbours under field conditions and thus increase the detrimental effects of this weedy species in crop fields; their evidence for this role of pollen is apparently also anecdotal. Although there have been reports of inhibitory compounds from acid fractions of *Pinus* pollen by Tanaka (1958, 1960, 1964a, b) and Sweet and Lewis (1971) (all cited by Stanley and Linskens, 1974) this paper and Sukada and Jayachandra (1980) appear to be the only descriptions of pollen allelopathy based on whole grains or aqueous extracts.

Importance in nature

We have stressed the rarity of interspecific transfer between *Hieracium* and *Diervilla* in nature, but this might not apply to all such interactions. In particular, bumblebees often show substantial species inconstancy when visiting superficially similar Compositae (Thomson, 1980), and there may be cases in nature where the intermingling of such species is great enough that such transfers may be routine (see Levin and Kerster, 1967; Waser, 1978b; Brown and Kodric-Brown, 1979). Less attractive species which depend on generalist pollinators must be especially at risk: Beattie (1969, 1971b) showed that insects visiting *Viola* in Britain always bore at least some foreign grains, and that transfer of *Endymion* and *Taraxacum* pollen to *Viola* stigmatic cavities occurred. Species which are highly attractive and occur in aggregations are less likely to suffer from such effects.

One caveat regarding the interpretation of these results concern the degree of mixing of the two pollens. The stirred mixtures applied by brush were probably more homogeneous than would be mixtures of the same overall composition applied by foraging bees, and this could inflate any inhibitory effect.

The prolific production of mostly 'useless' pollen by apomictic species (e.g. *Taraxacum* and *Hieracium*) is probably retained because of the advantages of occasional sexuality (Maynard Smith, 1978), but an additional incidental advantage of continued pollen production may exist in sabotaging the seed set of competing species (Faegri and van der Pijl, 1979). This study suggests that *Hieracium* pollen may have exceptional ability to play such an allelopathic role, but it is difficult to defend this function in terms of adapative significance. In a long-term evolutionary race between a saboteur and a victim, the victim's disadvantage (sexual reproductive failure) would seem to constitute a much stronger selection pressure than the saboteur's advantage (somewhat reduced competition for seed germination sites). However, weedy, successional colonizers like *Hieracium* may not often face long-term selective races, and it is possible that production of purely allelopathic pollen might be of some significance in the success of a clone entering into competition with an unpredictable collection of other weedy species for the occupancy of a limiting number of safe sites.

CONCLUSION

The ability of *Hieracium* pollen to inhibit successful fertilization in *Diervilla* is unexpectedly strong. The mechanism is unknown. Similar experiments should be conducted involving species whose pollens are likely to be mixed in nature. Since both documented cases of inhibitory pollen are from composites with allelopathic vegetative parts, it would be instructive to screen other such genera, e.g. *Helianthus* (Rice, 1975).

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